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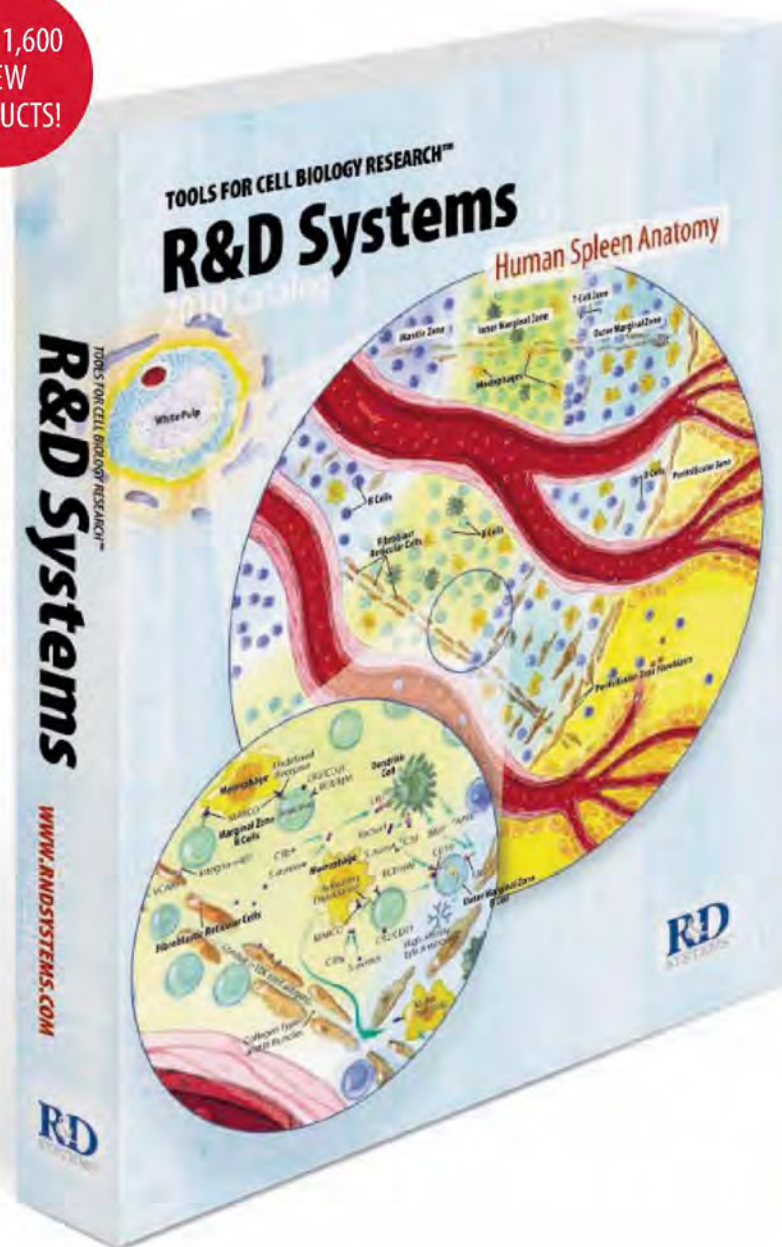
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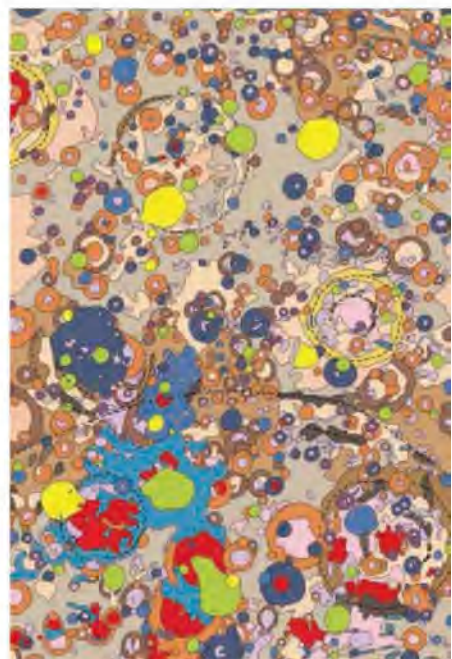
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Spore-bearing fungal parasites emerge from the digested corpses of three bdelloid rotifers. These freshwater invertebrates (length <0.5 millimeters) present an evolutionary puzzle because they have reproduced without sex for millions of years but have not been driven extinct by relentlessly coevolving parasites. Bdelloids can escape fungal parasites in space and time through complete desiccation and dispersal by wind to uninfected habitats. See page 574.

Image: Kent Loeffler, Kathie T. Hodge, Chris Wilson

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## SCIENCEONLINE

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[www.sciencexpres.org](http://www.sciencexpres.org)

### N-Terminal Acetylation of Cellular Proteins Creates Specific Degradation Signals

C.-S. Hwang et al.

In vivo protein stoichiometries are regulated through N-terminally acetylated degrons.  
10.1126/science.1183147

### Generating a Prion with Bacterially Expressed Recombinant Prion Protein

F. Wang et al.

Purified recombinant prion protein recapitulates the characteristics of the infectious agent in prion disease.  
10.1126/science.1183748

### Contributions of Stratospheric Water Vapor to Decadal Changes in the Rate of Global Warming

S. Solomon et al.

Decreases in stratospheric water vapor after the year 2000 slowed the rate of increase in global surface temperature.  
10.1126/science.1182488

### Symmetric Inertial Confinement Fusion Implosions at Ultra-High Laser Energies

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10.1126/science.1185383

## SCIENCENOW

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Highlights From Our Daily News Coverage

### Colliding Particles Can Make Black Holes

Simulations do not prove that the Large Hadron Collider will produce them, however.

### Birdlike Dinosaur Was Adept Glider

Foam model may clarify origin of bird flight.

### Hear That? Bats and Whales Share Sonar Protein

Similar genetic changes helped echolocation evolve in disparate species.

## SCENCESIGNALING

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### RESEARCH ARTICLE: Molecular Basis of the Death-Associated Protein Kinase–Calcium/Calmodulin Regulator Complex

I. de Diego et al.

The structure of the death-associated protein kinase and calmodulin complex reveals how calmodulin binding leads to kinase activation.

### RESEARCH ARTICLE: A Crucial Role for RACK1 in the Regulation of Glucose-Stimulated IRE1 $\alpha$ Activation in Pancreatic $\beta$ -cells

Y. Qiu et al.

RACK1 dictates the response of the intracellular stress sensor IRE1 $\alpha$  to different extracellular stimuli.

### PERSPECTIVE: Proteomics Modifies Our Understanding of Cell Cycle Complexity

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## PODCAST

M. Lafon and A. M. VanHook

A single amino acid substitution in a viral protein is sufficient to attenuate rabies virulence.

## SCIENCECAREERS

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### Perspective: From 'Pet' to Peer

S. Pfirman et al.

Diverse probationary faculty members may be denied a fair chance to become peers.

### Mind Matters: Gossip in the Lab

I. S. Levine

Workplace gossip may not be all bad, but it needs to be handled with care.

## Funding News

D. Adams

Get the latest announcements of research funding, fellowships, and internships.

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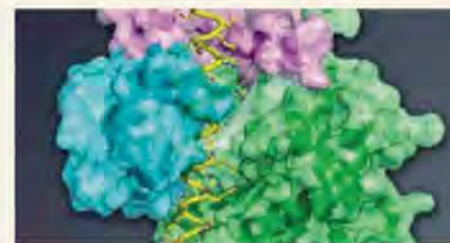
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### PERSPECTIVE: Pathogen Microevolution in High Resolution

R. K. Aziz and V. Nizet

Next-generation high-throughput sequencing provides insight into the origin and spread of microbial pathogens.



**SCENCESIGNALING**  
Structural insights into kinase activation by calmodulin.

### COMMENTARY: Transforming Medicine via Digital Innovation

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Digital devices have exceptional promise for changing the future of medicine.

### RESEARCH ARTICLE: Resiliency and Vulnerability in the HER2-HER3 Tumorigenic Driver

D. N. Amin et al.

The ability to overcome drug-resistant breast cancers may lie in dosage frequency.

### RESEARCH ARTICLE: Metabolomic Imaging for Human Prostate Cancer Detection

C.-L. Wu et al.

Metabolic imaging in the prostate reveals cancerous regions, guides biopsies, and helps prognosis.

## SCIENCEPODCAST

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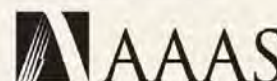
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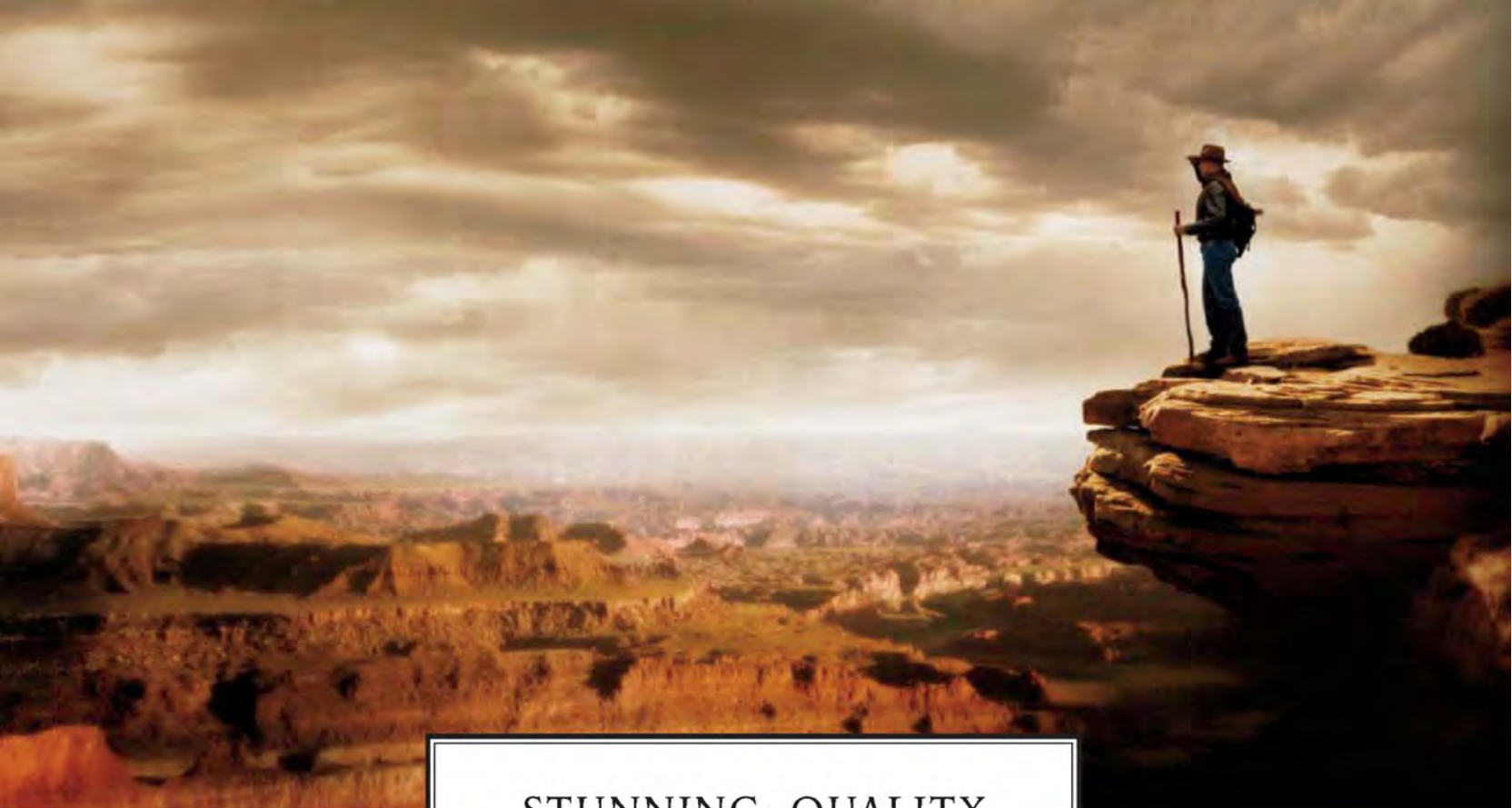
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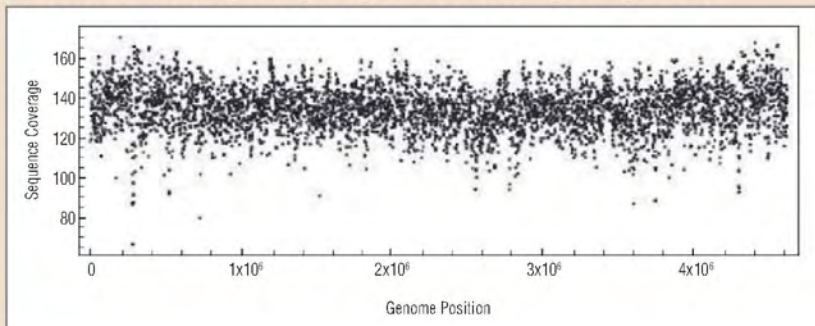


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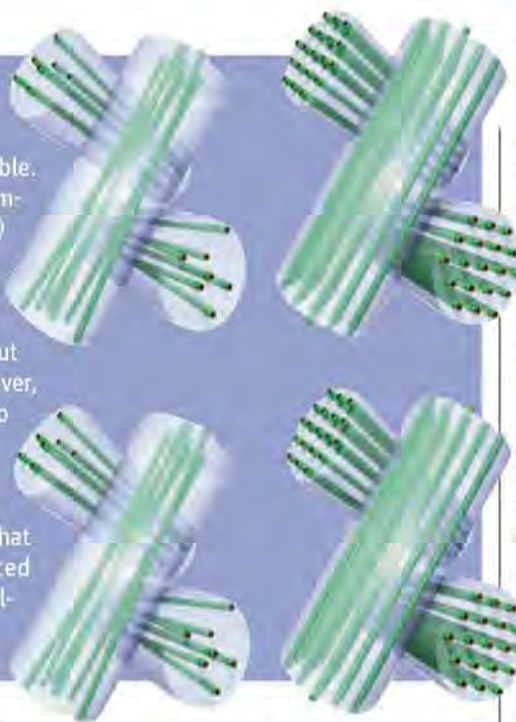
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## X-rays to Order >

Self-assembly of molecules is often irreversible. **Cui et al.** (p. 555, published online 17 December; see the Perspective by Safinya and Li) examined the ordering of a short peptide sequence (Ala<sub>6</sub>Glu<sub>3</sub>) terminated with an alkyl chain. Aqueous solutions of this molecule could form hexagonally ordered filaments, but more dilute solutions were disordered. However, prolonged x-ray exposure caused these arrays to become ordered. These arrays were stable for several hours but eventually returned to a disordered state; the addition of salts slowed the ordering processes. It is possible that during the ordering process, X-ray-induced charging affected the repulsive forces that balance tension within the filament.



## In the (Stem Cell) Zone

Efforts to isolate and characterize various types of stem cells have revealed much about the molecular mechanisms of stem cell self-renewal and differentiation, as well as the function of the microenvironment, or stem cell niche. **Li and Clevers** (p. 542) review what is known about stem cells in the hair follicle, bone marrow, and intestinal epithelium and suggest that two stem cell populations, one that is quiescent and one that is actively in the cell cycle, may coexist in these tissues in a so-called "zoned" stem cell model.

## Phase Transitions on Carbon Nanotubes

The nature of phase transitions changes with system dimensionality. Many aspects of two-dimensional systems have been explored by adsorbing rare gases on graphite surfaces. **Wang et al.** (p. 552) reduce the dimensionality further by examining phase transitions of argon and krypton on single-walled carbon nanotubes, following the extent of surface coverage and mapping out phase transitions by using the nanotube as a resonator. Changing the conductance and thus the density of surface electrons also allowed exploration of the effect of adsorbate-surface interactions.

## Packing Puzzle

The packing of a large number of spheres is a well-studied problem with maximal packing based on the arrangement of nearest neighbors. With much smaller numbers of particles, it is the free energy that governs which packing arrange-

ments dominate. **Meng et al.** (p. 560; see the Perspective by Crocker) looked at the assembly of colloidal clusters where the number of particles was limited from 2 to 10. For five particles or fewer, only one packing arrangement was found. For six or more particles, while a number of similar energy structures could form, the probability of formation was biased toward those structures with the greater number of nearest-neighbor connections.

## Big Finger

Alvarezsauroidea are an enigmatic group of theropods presumed to be closely related to birds, though most specimens are younger than *Archaeopteryx*. **Choiniere et al.** (p. 571; see news story by Stone) now describe a more complete early specimen, dating to about 160 million years ago, which supports the conclusion that Alvarezsauroidea are a basal group of the clade containing both birds and their close theropod relatives. The fossil also helps to reveal the evolution of this group's peculiar forelimb, which includes one enlarged functional finger.

## Sex Avoidance Strategy

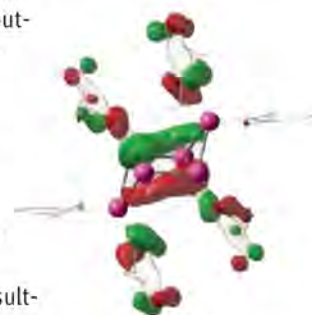
In metazoans, sex, as a means of reproduction, is almost universal, with popular theories suggesting that the high rate of genome mixing that sex promotes helps protect us from deleterious mutations and/or rapidly evolving parasites. Asexual organisms are both rare and often evolutionarily short-lived. If this rarity is due to failure to deal with parasites, then bdelloid rotifers, which have been abstaining from sex for millions of years, must be able to dodge the parasite bullet in some

other way. **Wilson and Sherman** (p. 574; see the cover) suggest that bdelloid rotifers do so by being both so resistant to desiccation and so amenable to dispersal on the air that their parasites can neither survive such punishing conditions nor spread as far nor as fast, allowing the asexual bdelloids to start afresh in (parasite-free) pastures anew.

## Aromatic Silicon

Benzene has long intrigued chemists on account of the energy stabilization, termed aromaticity, which arises from  $\pi$ -electron delocalization around its ring framework. A persistent question has been how such stabilization would be impacted were the carbons to be replaced by heavier atoms such as silicon.

**Abersfelder et al.** (p. 564) have prepared a benzene analog with Si atoms in place of all six-ring carbons, but a slightly altered bonding framework in which substituents outside the ring are no longer evenly distributed. Instead, the substituents pair up at two Si sites, leaving two other ring sites with no external appendages. The resulting compound no longer has a continuous network of  $\pi$ -electrons, but retains a degree of aromatic stabilization involving sigma and nonbonding electrons.



## The Best Things in Life Are Free?

Does money buy happiness? Answers to this question differ, depending, in part, on whether one asks an economist or a psychologist. The former would point to correlations between higher incomes and greater self-reported well-being, whereas the latter would argue that happiness shows little correlation with absolute material goods and is instead dictated largely by an individual's so-called set-point. Another strand of research invokes a hedonic treadmill, whereby income matters until subsistence requirements are met, at which point comparisons with one's neighbors are what influence one's sense of life satisfaction. **Oswald and Wu** (p. 576, published

*Continued on page 503*

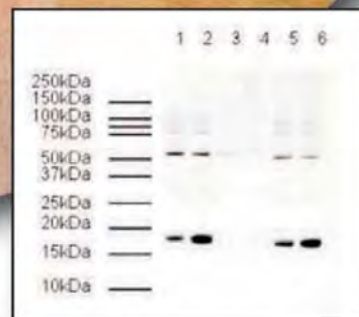




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online 17 December; see the Perspective by **Layard**) establish that the subjective responses from 1 million adults, collected within health surveys conducted by the U.S. Centers for Disease Control and Prevention, do indeed correlate with objective measures of quality of life.

## Platelet Microparticles Drive Inflammatory Arthritis

Platelets are best known for their critical role in blood clot formation during wound repair, but an appreciation for their role in inflammatory processes is growing. Platelet-derived cellular microparticles (MPs) are small membrane vesicles released from platelets in response to cell activation that can transport biomolecules throughout the body that have also been implicated in inflammatory processes. **Boilard et al.** (p. 580; see the Perspective by **Zimmerman and Weyrich**) have now found that platelet-derived MPs probably contribute to the inflammatory processes underlying rheumatoid arthritis, an autoimmune disease. The majority of MPs in synovial fluid from patients with various types of inflammatory arthritis were platelet-derived and, importantly, platelet-derived MPs were lacking in synovial fluid from osteoarthritis patients. Furthermore, platelet depletion abrogated disease development in a mouse model of inflammatory arthritis.



## Columns, Connections, and Correlations

What is the nature of interactions between neurons in neural circuits? The prevalent hypothesis suggests that dense local connectivity causes nearby cortical neurons to receive substantial amounts of common input, which in turn leads to strong correlations between them. Now two studies challenge this view, which impacts our fundamental understanding of coding in the cortex. **Ecker et al.** (p. 584) investigated the statistics of correlated firing in pairs of neurons from area V1 of awake macaque monkeys. In contrast to previous studies, correlations turned out to be very low, irrespective of the stimulus being shown to the animals, the distances of the recording sites, and the similarity of the neuron's receptive fields or response properties. In an accompanying modeling and recording paper, **Renart et al.** (p. 587) demonstrate how it is possible to have zero noise correlation, even among cells with common input.

## When Polymerases Collide

Genomes must be replicated by DNA polymerase and copied by RNA polymerase. The two processes occur concurrently in bacteria and, although the majority of bacterial genes are co-oriented with replication, head-on collisions still occur and can result in double-strand breaks in the genome. **Pomerantz and O'Donnell** (p. 590) show, in vitro, that both *Escherichia coli* DNA and RNA polymerases grind to a halt when they collide bumper to bumper but that the replication fork remains intact, with the RNA polymerase being shunted off the DNA and out of the way. Mfd, a transcription-repair coupling factor, promotes DNA replication restart probably by rewinding separated DNA stands ahead of the halted transcription complex, promoting its displacement from the DNA.

## Gut Check

The gastrointestinal (GI) tract is particularly sensitive to damage by ionizing radiation. Despite decades of study, fundamental questions such as which cells and which molecular mechanisms mediate this GI damage remain a source of great controversy. Studying a series of genetically manipulated mice, **Kirsch et al.** (p. 593, published online 17 December) conclude that GI epithelial cells, rather than endothelial cells, are the critical cellular targets of radiation damage and that apoptosis (a well-studied mechanism of cell death) is not a major contributor to the damage. Rather, an alternative cell-death pathway whose activity is inhibited by the tumor suppressor protein p53 appears to mediate GI damage. Further insights into this pathway may assist the development of medical countermeasures for preventing and treating radiation-induced tissue damage.

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# Call for Papers



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Bruce Alberts is Editor-in-Chief of *Science*.

## Science Education Web Sites

IN THIS ISSUE, WE ANNOUNCE THE FIRST OF 12 WINNERS OF A COMPETITION FOR WEB SITES THAT best promote science education. Each month this year, *Science* will publish an essay by the creators of a winning Web site that describes their online resource. This month's featured site focuses on teaching and learning genetics, and it originates from the University of Utah (see p. 538). The *Science* Prize for Online Resources in Education (SPORE) recognizes outstanding freely available online materials that enrich science education. There were nearly 100 entries for 2009 from many nations. They spanned diverse subjects, ranging from astronomy, chemistry, and physics to geology and biology. Most sites targeted students, ranging from elementary through graduate school, whereas others focused on the general public. Many included videos, animations, real-world data sets, or teaching materials.

A panel of 16 scientists and nine teachers performed the challenging task of selecting the winners from the excellent entries. In the end, two that were judged to be of the very highest quality were nevertheless not chosen. The Physics Education Technology (PhET) Web site, created at the University of Colorado, Boulder, was considered ineligible because *Science* had recently published an Education Forum that describes how to use PhET's physics simulations.\* In fact, this article provided the inspiration for the SPORE contest. An entry from the Howard Hughes Medical Institute (HHMI) was also not selected; although it produces an outstanding education Web site ([www.hhmi.org/biointeractive](http://www.hhmi.org/biointeractive)), HHMI is *Science*'s partner in producing the Education Forum, and we felt uncomfortable awarding them one of only 12 slots.

Why did *Science* create such a competition? There are many prizes for those who produce excellent scientific research, but only a few awards for educators. Yet being an outstanding science educator is as demanding and valuable to society as being an exceptional research scientist. And, as it does for research, highlighting education excellence sets a standard for others to aim at, while simultaneously emphasizing the enormous value of the endeavor. There is another important reason for the recognition that this competition brings. The World Wide Web is a fantastic information resource, but it can be overwhelming. Many had hoped, for example, that the U.S. National Science Digital Library Project might go a long way toward solving this problem.\*\* But the collection of science education Web sites that resulted, although a valuable resource, contains so many entries that additional guidance seems warranted. With a limit of 12 Web sites a year, *Science* aims to make it easier to find valuable materials, both for one's intellectual growth and for teaching.

This last point raises a broader issue. When I began my academic career as an assistant professor at Princeton University in 1966, I sought to learn everything about what others had discovered previously, before beginning my research on chromosome replication. Yet when I taught, I rarely sought to build on what other teachers had developed before me. This difference between how scientists approach their research and their teaching goes a long way, I believe, to explain why the quality of university science education lags so far behind the quality of science itself.

Through the Web, a rapidly expanding OpenCourseWare Consortium, with more than 150 universities from 36 nations, makes different approaches to teaching readily observable globally. Based on this wide visibility, many more contests can be developed to reward innovation in science education. Scientific societies might, for example, annually recognize the best 1-month teaching modules for an introductory science course in college, or provide an award for the best set of laboratory modules for a science class that are inquiry-based and require only modest resources (thereby being readily exportable). The nomination process for *Science*'s 2010 SPORE contest has just begun ([www.aaas.org/go/spore](http://www.aaas.org/go/spore)). According to Wikipedia, a "spore is a reproductive structure that is adapted for dispersal and surviving for extended periods of time in unfavorable conditions." Analogously, we hope that SPORE seeds the proliferation of many other education awards, adapted for dispersal and survival in the world of education.

— Bruce Alberts

10.1126/science.1187267

\*C. E. Wieman et al., *Science* 322, 682 (2008). \*\*J. Mervis, *Science* 323, 54 (2009).



## MICROBIOLOGY

## Working Backwards

What do you get when you throw anti-idiotypic antibodies into the Sargasso Sea? Well, probably not even a tiny splash, but conceptually what can be harvested is a bounty of previously undescribed and unsuspected virus genomes. When viruses infect plants, the plants fight back by generating an immune response that makes use of and is tailored to the specific virus; small RNAs, roughly 21 to 25 nucleotides in size, are synthesized by the host and used to target and destroy complementary viral RNAs. In developing a diagnostic method for two sweet potato viruses that cause corky lesions in the roots, Kreuze *et al.* discovered that it was possible through deep sequencing technology to recover the viral genomes by assembling the overlapping sequences of the small RNAs produced by infected plants. In addition to the expected culprits, they identified new members of the badnavirus and mastrevirus genera, which contain species that infect banana and sugarcane plants, respectively. Wu *et al.* have analyzed libraries of small RNA sequences derived from the fruit fly and the mosquito, and they were also able, via deep sequencing and metagenomic analysis, to identify the presence of a number of new viruses, whose complete genomes could then be reconstructed by PCR. — GJC

*Virology* **388**, 1 (2009); *Proc. Natl. Acad. Sci. U.S.A.* **107**, 10.1073/pnas.0911353107 (2010).



## CELL BIOLOGY

## Meet U at the Terminal

Parkinson's disease is characterized by progressive neurodegeneration and has been linked to mutations in the gene *parkin*, which encodes a protein that recruits enzymes that catalyze the conjugation of ubiquitin to target substrates. Parkin itself contains a ubiquitin-like (Ubl) domain, which is structurally similar to ubiquitin but is not rich in proline residues. The Src homology 3 (SH3) domain is found in hundreds of copies in the human proteome, and it was originally discovered as a mediator of protein-protein interactions, particularly via its affinity for proline-rich regions. Trempe *et al.* have found that the Ubl domain in parkin binds to the SH3 domain in endophilin A, which is involved in the retrieval of synaptic vesicles during neuronal activity. Phosphorylation increased the interaction of parkin with endophilin A, and also stimulated the ubiquitination of a group of proteins in synaptic nerve terminals in wild-type mice, but not in parkin-deficient animals. Thus, a regulated interaction between ubiquitination and endocytosis may provide a clue to the pathophysiology in patients with Parkinson's disease. — HP

*Mol. Cell* **36**, 1034 (2009).

## MICROBIOLOGY

## Living Off Anesthetic

Toxicity concerns have largely eliminated chloroform's erstwhile use as an anesthetic, though the

compound still plays a role in industrial chemistry. Low levels of chloroform (trichloromethane) inhibit the metabolisms of bacteria responsible for detoxifying many other chlorinated hydrocarbons; therefore, its persistence in the environment not only presents a possible human health risk but also slows down natural remediation processes. Now, however, Grostern *et al.* have discovered a population of *Dehalobacter* that can not only tolerate a high level of chloroform but actually grow on it by coupling dechlorination to respiration. Kinetics studies suggest that the bacteria use a reductive dehalogenase enzyme specific to chloroform and the structurally similar trichloroethane. Direct reduction of chloroform coupled to bacterial growth is advantageous for remediation because it is more efficient and does not require the addition of separate growth substrates. Contaminated aquifers—such as the one from which these bacteria were originally isolated—may be fertile grounds for other bacteria with specialized adaptations to tolerate toxins. — NW

*Environ. Microbiol.* **12**, 10.1111/j.1462-2920.2009.02150.x (2010).



## APPLIED PHYSICS

## Plasmons on Separate Paths

In the quest for faster signal processing speeds there are essentially two approaches: shrink the size of the circuitry, or use light rather than electronic current as the information carrier. In the former approach, the operating frequency faces a fundamental upper limit of several tens of GHz due to the properties of the materials required in the design of current-based devices. Light, in contrast, offers the ultimate speed, but requires circuit size to remain on the order of its roughly micrometer-scale wavelength. Surface plasmons—light-induced electronic excitations near the surface of a metal/dielectric interface—offer the possibility of exploiting the speed and size virtues of the two approaches. However, exciting the plasmons is usually a resonant phenomenon, meaning that only one kind of plasmon can be excited at a particular wavelength. Williams *et al.* present a technique based on a designed array of annular holes on a textured copper surface that allows two tightly bound THz-induced plasmon modes to propagate independently, thus providing potential for a number of applications in chemical and biological sensing, security screening, and communications. — ISO

*Appl. Phys. Lett.* **96**, 011101 (2010).



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## LOADING SPRINGS



Village in Uttarakhand;  
(inset) dam collects rainwater.

Springs are a major source of water in Himalayan villages. But spring water has become scarcer, even drying up in summers because of deforestation and changes in rainfall. Harvesting rainwater can help, but convoluted mountain topography often hides the recharge areas where precipitation goes underground to feed the springs below.

To find those areas, scientists are turning to radioisotopes. A team headed by physicist K. Shivanna of the Mumbai-based Bhabha Atomic Research Centre (BARC) took samples of rainwater and spring water from various locations. The isotopic composition of rainwater varies by season, latitude, altitude, and distance from the coast; the differences leave their mark on water flowing from springs.

In a 2-year pilot project, the researchers and a local group, the Himalayan Environmental Studies and Conservation Organization (HESCO), compared water samples in the Alaknanda River Basin in the Himalayan state of Uttarakhand in India. From isotope signatures of the rainwater in the upper reaches and the water in the various springs, the BARC team identified possible recharge zones for the springs in the three valleys. "This made it possible to accurately locate dams, trenches, and underground dikes to give rainwater time to seep into the springs below," says HESCO founder Anil Joshi. As a result, the discharge rate of existing springs almost doubled and two new springs appeared. Shivanna says there are now plans for similar projects in seven other Himalayan regions.

## Hormesis Who?

Many biologists have noticed that when they feed a plant or animal a tiny amount of a poison, the result is the opposite of what they see at higher doses—the plant grows faster, for example, or the mouse develops fewer tumors than it normally would. That "biphasic" phenomenon is known, among other terms, as

hormesis. Mainstream scientists have mostly ignored such observations (*Science*, 17 October 2003, p. 376). But lately, hormesis has been gaining ground, especially in Asia. A search of the Web of Science database finds that citations of papers using the term "hormesis" or "hormetic" have soared from about 200 in 1999 to more than 2400 in 2009. Toxicologist Edward Calabrese of the University of Massachusetts, Amherst, who did the search, predicts that citations "will profoundly grow" as people realize the implications of hormesis in areas such as finding new anticancer drugs.

## Baby Einstein Goes to Court

The co-founder of the company that introduced the popular Baby Einstein videos has sued to obtain the records of researchers whose work cast doubt on the value of the series.

William Clark, who founded The Baby Einstein Co. with his wife in 1996, sued the University of Washington this month for access to materials held by UW pediatric researchers Dimitri Christakis and Frederick Zimmerman. The pair wrote two widely publicized studies in 2004 and 2007: One suggested that TV-immersed toddlers may be at risk for later attention problems; the other linked watching baby videos with delays in language development (*Science*, 24 August 2007, p. 1015).

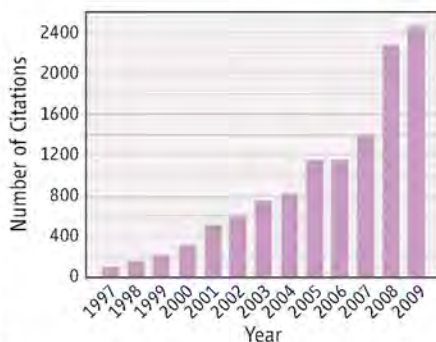
"Given that other research studies have not shown the same outcomes, we would like the raw data and analytical methods from the Washington studies so we can audit their methodology," Clark said in an 11 January statement. The Clarks sold their business to Disney in 2001.

Both papers were influential in deflating educational claims used to market baby videos, says Josh Golin of the Campaign for a Commercial-Free Childhood, which in 2006 complained to the Federal Trade Commission about Baby Einstein. Disney has since toned down its claims. And last September, facing the threat of a class-action suit, the company announced that it would give refunds to dissatisfied parents. "It's a brand trying to save itself," Golin says.

## Old Man of the Sea

Oceanographer Walter Munk, 92, has won the 2010 Crafoord Prize for planet-spanning work explaining everything from why Earth wobbles to how the wind moves ocean waters. The prize, with a \$555,000 award, comes 10 years after he won the Kyoto Prize, the other major honor billed as a Nobel equivalent. "I'm so delighted about both," he says. "I thought I was beyond that."

Not many scientists have authored papers "still cited 70 years later," says fellow physical oceanographer Carl Wunsch of the Massachusetts Institute of Technology in Cambridge. "He has a knack of getting to the heart of one subject after another." Munk, who still drives to his office at the Scripps Institution of Oceanography in San Diego, California, almost daily, is now working on a neglected sort of wave that he thinks is vital to driving ocean currents. Then there's a sort of 3D x-ray of the ocean he wants to apply to addressing sea-level rise. But that, he says, "is a hope for the future."







## PALEONTOLOGY

## Bird-Dinosaur Link Firmed Up, And in Brilliant Technicolor

**BEIJING**—Which came first, the chicken or the dinosaur egg? That one's a cinch. Less obvious is the riddle of kinship. Most scientists think birds evolved from dinosaurs about 150 million years ago. But a sparse fossil record has provided ammunition to those who insist that birds arose independently. A stunning new fossil makes that idea virtually untenable. And a second paper this week brings dinosaur feathers vividly to life, offering new clues to why this instrument of flight first evolved in flightless creatures.

On page 571, fossil-hunter extraordinaire Xu Xing of the Institute of Vertebrate Paleontology and Paleoanthropology (IVPP) here and colleagues unveil *Haplocheirus sollers*, a new genus of alvarezsauroid—a group of dinosaurs once thought to be flightless birds. The nearly complete skeleton, unearthed from 160-million-year-old mudstone deposits in northwestern China's Junggar Basin, extends the fossil record of alvarezsauroids back in time by a whopping 63 million years—making it about 15 million years older than the earliest known bird, *Archaeopteryx*.

In 2006, specimens from another theropod group—tyrannosauroids—challenged the so-called temporal paradox: the fact that

irrefutable birdlike dinosaurs appear millions of years after *Archaeopteryx* in the fossil record. (Among other birdlike features, tyrannosauroids sport three-toed feet, hollow bones, and even wishbones.) Along with those and other recent finds, *Haplocheirus* “establishes once and for all that there is no temporal paradox,” asserts Hans-Dieter Sues, curator of vertebrate paleontology at the National Museum of Natural History in Washington, D.C.

The newest, oldest alvarezsauroid dispels some of the group's mystique. “Alvarezsauroids were collected for the first time in the 1920s but were so enigmatic that they were never recognized and described until the 1990s,” says Philip Currie, a paleontologist at the University of Alberta in Edmonton, Canada. As specimens turned up in Asia, Europe, and North America, debate raged over whether they represented dinosaurs or flightless birds. *Haplocheirus* “definitely settles this dispute,” Sues says. The exquisitely preserved specimen “is so primitive,” says IVPP's Zhou Zhonghe, that it lacks some birdlike features seen in later alvarezsauroids such as fused wrist bones, a backward-facing pubis, or a crest near the top of the shinbone.

**Birds of a feather.** A new avianlike alvarezsauroid (below) predates the first bird, *Archaeopteryx*—powerful evidence that birds arose from dinosaurs. Fossilized pigment organelles reveal the true colors of *Sino-sauropteryx* (left).



The controversy highlights how hard it can be to distinguish birds from dinosaurs. “Deep in evolutionary history, it's extremely difficult to draw the line,” Xu says. The discovery of feathered dinosaurs in the late 1990s turned classification upside down.

Why feathers evolved in dinosaurs is a puzzle. One idea is that feathers provided insulation. Another is that they evolved as camouflage or to attract a mate. But evidence such as distinctive colors or patterns had eluded researchers—until now. This week in *Nature*, paleontologist Mike Benton of the University of Bristol in the United Kingdom and colleagues offer the first report of organelles bearing the pigment melanin in dinosaurs. They found melanosomes in the theropods *Sinornithosaurus* and *Sino-sauropteryx* and in a bird, *Confuciusornis*, that lived roughly 125 million years ago.

Although many scientists expected dinosaur feathers to contain melanosomes, finding them wasn't easy. In Benton's lab in Bristol, IVPP's Zhang Fucheng says he “spent day and night for more than a year” studying scanning electron microscope images of scores of fossil specimens. Zhang's diligence paid off—he uncovered melanosomes containing the two most common kinds of the pigment: eumelanin, which gives a gray or black tinge, and pheomelanin, which gives a chestnut to reddish-brown color. The find could settle a debate over whether bristles studding *Sino-sauropteryx* were primitive feathers or collagen fibers. “Melanosomes prove that those structures are indeed feathers,” Benton asserts.

The discovery also reveals the true colors of dinosaurs. *Sino-sauropteryx*'s protofeathers ran in alternating orange and white rings down its tail like a barbershop pole. To Benton, the vibrant pattern suggests that “feathers first arose as agents for color display and only later in their evolutionary history did they become useful for flight and insulation.” Others are not so sure. Zhou, a co-author of the *Nature* paper, still thinks feathers evolved for insulation.

To Xu, the mystery has deepened. “Now we are realizing that feather evolution is more complex than we thought,” he says.

—RICHARD STONE

CREDITS: (LEFT) ARTWORK BY CHUANG ZHAO AND LIDA XING; F. ZHANG ET AL., NATURE (ADVANCED ONLINE EDITION) © 2010 NATURE PUBLISHING GROUP; MACMILLAN PUBLISHERS LIMITED, (RIGHT) PORTIA SLOAN





Toward quantum machines

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Curing diabetes in South Dakota

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## GLOBAL HEALTH

# Haiti's Quake Shifts Clinic's Focus From AIDS to Aid

On one and a quarter hectares in downtown Port-au-Prince, the AIDS clinic called GHESKIO has developed a reputation over the past 25 years as a place that, improbably, provides thousands of Haitians with free care and conducts world-class clinical research. Led by Jean "Bill" Pape, GHESKIO (the Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections) has continued to function since the 12 January earthquake. The clinic has now become one of the few places where locals can receive emergency care in that part of the devastated city and has converted itself into a hospital with a surgical unit and a makeshift outdoor home for thousands of refugees.

GHESKIO collaborates with the Center for Global Health at Weill Cornell Medical College in New York City, and together they receive about \$3 million a year from the U.S. National Institutes of Health to conduct HIV/AIDS clinical research and training. All of that has now been put on indefinite hold, as Pape and his staff of more than 300 people work to save the lives of those who have nowhere else to turn—while they still keep delivering HIV/AIDS and tuberculosis testing, counseling, and treatment to more than 100,000 others who each year regularly visit the clinic and its newer satellite near the airport. In many ways, GHESKIO is Port-au-Prince's equivalent of Partners in Health, the nonprofit program run by Harvard Medical School's Paul Farmer that serves rural Haiti.

Pape was in a meeting with the prime minister, minister of health, and officials from the World Health Organization and other international groups when the magnitude-7.0 earthquake hit. All escaped from the room, which collapsed, relatively unscathed. On 21 January, he responded to questions from *Science* in an e-mail; his responses have been edited and condensed.

—JON COHEN

**Q: What is the status of your staff?**

**J.P.:** The earthquake affected us a lot. We have documented the loss of 4 staff members



**Helping hands.** GHESKIO head Pape converted his downtown Port-au-Prince AIDS clinic into a refugee camp and emergency hospital complete with a surgical unit.

including 1 MD and the head of the microbiology lab, and 12 injured staff. Twenty-one people have not reported to work.

**Q: What damage did your clinics suffer?**

**J.P.:** Three important buildings are severely damaged, two at one site and one at another. The other buildings can be repaired. [In an earlier e-mail to colleagues at Cornell, Pape noted that GHESKIO's tuberculosis lab had to be sealed until it is decontaminated, as tubes containing multidrug-resistant TB bacteria and other strains had broken.]

**Q: What stockpile do you have of antiretroviral drugs [ARVs] and are you in danger of running out?**

**J.P.:** Our AIDS care program is going well. We activated our contingency plan the morning after the earthquake. This was easy as we have been used to unstable political situations and hurricanes. Basically, all patients have an extra supply of 2 weeks of important drugs (ARVs and TB).

**Q: Are you able to distribute ARVs to the people most in need?**

**J.P.:** We are now seeing about 80% of all patients we were seeing before. PEPFAR [the U.S. President's Emergency Plan for AIDS

Relief] personnel were delivering ARV drugs even during the aftershocks.

**Q: What assistance are you receiving?**

**J.P.:** We have the full back-up of both U.S. Ambassador Eric Goosby, who even volunteered to come and help us, as well as Michel Kazatchkine, executive director of the Global Fund and his entire staff. They have found another way to help us through

COPRESIDA, the agency in charge of the AIDS program in the Dominican Republic. This catastrophe has revealed that the DR is a true friend of Haiti. We are grateful for the world response and particularly that of DR and the personal involvement of President Leonel Fernández.



**Q: How do you restart the clinical trials and training you had under way?**

**J.P.:** We have stopped enrolling new volunteers in study projects, and we have stopped training activities. We have two new missions: Providing humanitarian aid relief to 5000 refugees who are on our premises after the earthquake—and need everything—and taking care of the injured.

**Q: Who else is working with you?**

**J.P.:** This new mission has strengthened our collaboration with Quisqueya University to educate the population and avoid a major epidemic of waterborne infectious diseases. We're also collaborating with other new partners, such as World Water [Relief] to provide potable water and Action contre la Faim to set up latrines. The U.S. Department of Health and Human Services has supported us in setting up a complete hospital able to handle all medical and surgical emergencies.

**Q: How are you holding up?**

**J.P.:** We did not choose this situation and it's tough, but we are up to this new challenge.





**In the hot seat.** IPCC chair Rajendra K. Pachauri rebuts allegations of sloppy science and financial conflict.

## NEWSMAKER INTERVIEW

# Climate Science Leader Rajendra Pachauri Confronts the Critics

**NEW DELHI**—It has been a long, hot winter for the Intergovernmental Panel on Climate Change (IPCC) and its chair, Rajendra Kumar Pachauri. E-mails leaked last November cast doubt on the integrity of a few of the 4000 scientists who produce consensus reports for the U.N. body on climate change science (*Science*, 4 December 2009, p. 1329). Then IPCC earlier this month offered regret for having included an unsupported prediction in its fourth assessment in 2007 that Himalayan glaciers would melt away by 2035 (*Science*, 13 November 2009, p. 924).

Pachauri, a 69-year-old industrial engineer and director general of The Energy and Resources Institute (TERI) here, has headed IPCC since 2002. He routinely puts in 18-hour days and is not known to have taken a vacation in 3 years. The workaholic has recently come under attack in the U.K. press for his lucrative stints as an adviser to companies including the Toyota Motor Corp. and Deutsche Bank—earnings that he insists go to TERI. On a cool, smoggy morning here earlier this week, Pachauri defended IPCC's work and shot back at critics who want to see him ousted as panel chair. —PALLAVA BAGLA

**Q: The big issue dogging IPCC this winter is the inclusion of a prediction in the fourth assessment that Himalayan glaciers would melt by 2035. IPCC has offered regret—but not an apology.**

**R.K.P.:** We have made a mistake and we have admitted that. Our job is essentially to bring the science into our assessments from the best sources that exist. Look at the extent of the glaciology work that has been done in this country. It is pathetic. I mean, that is really where we need to come up with an apology.

**Q: In a 20 January statement, IPCC still says that India's glaciers are melting away. Isn't that a tall claim?**

**R.K.P.:** Our glaciers are under the same influences, the same temperature changes as other glaciers in the world. So you know we cannot possibly assume if all the other glaciers are melting, that for some reason we are insulated from those influences. The lay public ... can see with their eyes what is happening to our glaciers.

**Q: What is your stance on linking global warming with extreme events? Has IPCC made a blunder by suggesting the link?**

**R.K.P.:** No, we have not made a blunder, and we are going to issue a statement on that. We decided well over a year ago to do a special report on climate change and extreme events. We would like to assess all the new information and research now available.

**Q: Some critics contend that while IPCC was projecting that it was doing great science, it is turning out to have done some sloppy work.**

**R.K.P.:** While I am sure there are some people who believe that, I also can tell you that there is a large body of people who look at the entirety of what IPCC has done. We have placed before the world ... a defining piece of work, which clearly tells you about the scientific reasons for climate change.

The veracity, the honesty, the scrupulousness with which we carry out our assessment has been the hallmark of the IPCC, and we are never going to compromise on that.

**Q: What have you learned from these episodes?**

**R.K.P.:** We have got to ensure that all our procedures are followed in letter and spirit and with a huge amount of due diligence. I will personally make sure that all the lead author teams that are going to work on the fifth assessment report and our special reports observe this scrupulously, go the extra mile in making sure that we don't use any information that is questionable. What has happened only highlights the importance of the procedures that we have established. If they had been followed, we wouldn't have got into this unfortunate error.

**Q: The other issue that dogged IPCC is the leaked e-mails from the [Climatic Research Unit of the University of East Anglia in Norwich, U.K.].**

**R.K.P.:** Those e-mails represent nothing more than private communications, private airing of anguish or anger or emotion. It was indiscreet.

**Q: Has all that has happened this winter dented the credibility of IPCC?**

**R.K.P.:** I don't think the credibility of the IPCC can be dented. If the IPCC wasn't there, why would anyone be worried about climate change?

There are those who would wish to demolish the science of climate change. Our vindication will lie in our performance.

**Q: Are you being made a fall guy?**

**R.K.P.:** I am not a fall guy, but you know the buck stops here. I am the chairman; I am not going to shirk responsibility.

**Q: Is there a conflict of interest between your role as IPCC chair and your work advising companies?**

**R.K.P.:** I don't see any conflict at all. Science has to be used for decision-making. IPCC's work is supposed to be very clearly policy relevant. How can I establish policy relevance if I shut myself in an ivory tower and say I will not say anything about climate change? I feel totally comfortable in the role of adviser to anybody.

CREDIT: SHEN HONG/XINHUA/LANDOV



**Q:** A statement from TERI lists the number of companies you are associated with, the money which has flowed back to you and the organization: €100,000 from Deutsche Bank, \$80,000 from Toyota, and so forth. You don't think this is conflict of interest?

**R.K.P.:** Where is the conflict of interest? I am a paid employee of my institute, not of the IPCC. I don't see why I shouldn't advise anybody anywhere in the world ... as long as I am not making money out of it. [The money] is going to my institute.

**Q:** Some people disagree; they believe that

you have to be cleaner than Caesar's wife.

**R.K.P.:** Yeah, but Caesar was also murdered by Brutus, wasn't he? Caesar was murdered by a group of people for their own interest, all right? So I cannot possibly be held accountable for all the lies that the media are writing about in a certain section of the U.K. press. I mean, if they are going to influence public opinion, I can assure you it is not going to last forever. I am absolutely convinced the truth will prevail in the end.

**Q:** You put up a brave face, but some in the scientific community feel let down. They say

that you are carrying too much baggage, that it's time for you to move on.

**R.K.P.:** I certainly have no intention to quit. I will continue as the chairman of the IPCC till I have completed the fifth assessment report.

**Q:** Are you becoming a thorn in the side of vested interests—a thorn they wish to eliminate?

**R.K.P.:** No question about that. But I have no intentions of backing off. I am not going to tailor the truth to suit the vested interests of those who would like to continue with business as usual.

## PHYSICS

# NRC Urges U.S. to Rethink Sale of Helium Reserve

In 1996, the U.S. Congress decided to sell the 1 billion cubic meters of gaseous helium—specifically the heavier isotope, helium-4—that the country had stockpiled. But conditions it imposed on the sales are keeping the price of helium artificially low and encouraging waste of a substance indispensable for numerous scientific and technological applications, says a National Research Council report released last week.

"Helium is being sold at fire-sale prices, and low prices are not going to encourage the recycling, conservation, and substitution that might prolong the existing supply," says Charles Groat, a geologist at the University of Texas, Austin, and co-chair of the committee that wrote the report.

Produced in radioactive decay, helium collects in the same rock formations that trap other gases and is primarily a byproduct of the natural gas industry. It is the only element that remains a liquid at absolute zero, making it an unparalleled cooling agent, or "cryogen." Without helium, the superconducting magnets in MRI machines won't work and myriad lines of physics research would grind to a halt. Helium is also essential to purge the tanks and lines in rockets that burn liquid hydrogen.

In 1960, Congress told the now-defunct Bureau of Mines to stockpile helium piped from gas fields in Kansas, Oklahoma, and Texas in a rock formation called the Bush Dome Reservoir near Amarillo, Texas. By 1973, the dome held 1 billion cubic meters of gas. But the bureau's helium sales were weaker than expected, and the reserve was losing money. So 13 years ago, Congress told the Bureau of Land Management (BLM), which had taken control of the helium, to sell almost all of it by 2015.

Congress required BLM to sell the gas for

enough money to pay off the reserve's debt—\$1.66 per cubic meter with increases for inflation. At the time, BLM's price for crude helium was above the market price for refined helium. Since 1995, however, global demand for helium has increased by nearly 70%, and BLM's current price of \$2.29 per cubic meter is below the price from private sources.

The 60 million cubic meters pumped from the reserve each year make up half the crude helium brought to market in the United States

for big consumers such as NASA and the Department of Defense that would ensure a supply in times of shortage, the report says.

The report even suggests that Congress rethink the sale of the reserve, as the world's resources could be depleted within 40 years and demand could exceed supply within a decade. "Probably 10 or 15 years ago it was heresy to say we need a reserve," Groat says. "Now that the situation has changed, I think that may be revisited." At the least, he says, Con-

## HOW THE U.S. USES HELIUM



**Wise use?** Helium is indispensable for chilling the superconducting magnets in the Large Hadron Collider (right) and manufacturing optical fibers, but not for welding and filling balloons.



and a third of the total worldwide. So, the report says, the low price, which BLM sticks to as a matter of policy, drives the market and spurs needless consumption, such as the 15 million cubic meters used annually by welders in the United States. (Europeans use argon.)

BLM should establish a higher market-based price, the report says, although that may be tricky, as only four refiners have access to the pipeline to the dome. To soften the blow to scientists, those with grants from agencies such as the National Science Foundation, the National Institutes of Health, and the Department of Energy should be allowed to buy BLM helium under terms currently reserved

gress will have to tell BLM what to do after 2015, as the bureau will miss the deadline for selling the remaining 650 million cubic meters of gas by years.

Will Congress heed the report? Maybe, says one congressional staffer. An acute shortage of the lighter isotope of helium, helium-3, has already grabbed legislators' attention, he says, because it may derail the Department of Homeland Security's plan to deploy thousands of helium-3-filled radiation detectors (*Science*, 6 November 2009, p. 778). "At least you can say to members, 'You were working on this, and here's this other part of the problem you should be aware of.'" —ADRIAN CHO



# In Central California, Coho Salmon Are on the Brink

Lagunitas Creek starts high on the north flank of Mount Tamalpais, just north of San Francisco, California, and makes a short run to the Pacific Ocean, passing through a rural valley and a coastal redwood forest. It was once a thriving breeding ground for coho salmon. Local legends tell of streams so thick with fish returning from the sea to spawn that a person could walk from one side to the other on the fishes' backs. The state record coho, a 10-kilogram whopper, was caught on a tributary in 1959.

But those days are long gone. The subspecies of coho that lives along the central California coast is the most endangered of the many troubled salmon populations on the West Coast of North America, says Charlotte Ambrose, a recovery coordinator with the National Marine Fisheries Service (NMFS) in Santa Rosa, California. Listed as an endangered species in 2006, the cen-

tral coast coho's numbers have recently taken an even sharper turn for the worse. As this year's winter spawning season draws to an end, biologists who've been surveying streams and rivers throughout the fish's range are reporting dismal numbers. A federal species recovery plan to be released next month has morphed into a species survival plan, Ambrose says: "We truly are at the brink of extinction."

The recovery plan will focus on 28 watersheds where NMFS thinks habitat restoration efforts—such as restoring floodplains, preserving forested areas along creek sides, and placing woody debris in streams to provide shelter for fish—can have an immediate impact on the coho's survival. Lagunitas Creek, which has one of the strongest remaining runs of wild central coast coho, is one. A tour of the watershed last week illustrated why it may be one of the coho's last best hopes—and why success is far from guaranteed.

In a soaking rain, Greg Andrew, a fishery program manager with the Marin Municipal Water District, unlocked a gate and piloted his hybrid SUV up a steep, unpaved road that parallels the creek. After a kilometer, what look like two giant concrete slides come into view: spillways for Peters Dam, the largest of seven dams built in the area between 1872 and 1979 to create drinking water reservoirs. The dams blocked off about half of the former coho habitat, Andrew says: "We're trying to make what's left as good as possible."

These efforts include periodic dips in the road and other drainage features added to reduce the amount of sediment that washes into the creek, where it can suffocate salmon eggs and clog nooks and crannies in the streambed that young fish use for shelter. Further downstream, Andrew points out a woody debris structure in the creek, one of about 60 built by the water district. These strategically placed piles of logs create slow eddies where fish can escape the raging flows created by winter storms.

The final stretch of Lagunitas Creek passes through Point Reyes National Seashore before emptying into Tomales Bay. In a \$6.2 million project completed in 2008, the National Park Service knocked down levees at the mouth of the creek and restored more than 100 hectares from cattle pasture into a tidal wetland. The project provides crucial floodplain habitat for coho and other



CREDITS: (SOURCE) NOAA; (PHOTO INSET) MORGAN BOND/NOAA/SWFSC



wildlife, says park service hydrologist Brannon Ketcham. For coho, the wetlands provide another refuge from the rushing water in winter and a place for smolts to bulk up before heading out to sea in spring.

If only there were more fish to take advantage of it. As of last week, biologists surveying the creek for the Marin water district had counted only 64 coho in Lagunitas Creek and its tributaries. In the already lean years prior to the coho's addition to the endangered species list, nearly 600 fish returned on average. Because the run usually peaks in early January, Andrew doesn't expect the count to rise substantially, if at all. Across the entire range, Ambrose estimates that only 500 fish have returned this year.

In Lagunitas Creek and elsewhere, this marks the third straight year of abysmal returns for the coho, an especially ominous milestone, biologists say, because of the fish's 3-year life cycle. Salmon born in a given year migrate out to sea and return to their natal stream 3 years later to spawn and die. This creates three distinct "year classes," each of which returns every 3 years. This year's feeble return suggests that all three classes are faring poorly across the coho's range.

The precipitous decline is probably due to a combination of factors, beginning with a 150-year history of land and water use—including dams, mining, agriculture, and urbanization—that have degraded freshwater habitat throughout the central coast coho's range. More recently, the seasonal upwelling of nutrient-rich deep water off the California coast has been delayed, which may have reduced the availability of food for hungry smolts beginning their migration out to sea, says Brian Spence, a research fishery biologist with NMFS in Santa Cruz. Moreover, California's 3-year drought has dried up streams and delayed access to spawning grounds, he says: "When you stack all that on top of the already diminished freshwater capacity, I think that's why some of these populations are getting precariously close to blinking out altogether."

Conserving and improving what's left of the coho's freshwater habitat is the best hope for the fish's survival, says Ambrose. Lagunitas Creek illustrates the best case scenario, she adds. Everyone from the National Park Service to the county water district to local conservation groups has done work on the coho's behalf. "There is nothing like this kind of collaboration anywhere else in the range of the central coast coho," Ambrose says.

But even here there is some resistance. At an animated public meeting last week,

some residents voiced opposition to a salmon conservation plan commissioned by the county government on the grounds that it could violate their property rights. More than 100 people braved the rain and packed into a room at a local school. As a woman from the environmental consulting firm that prepared the plan explained one of its main recommendations—preserving an undeveloped buffer zone of up to 30 meters along the sides of the creek—many in the audience groaned. In a narrow valley where many lots hew closely to the creek, the idea did not seem to sit well. One man yelled out: "Are you being realistic? Are you on planet Earth?" In the public comment session, residents' concerns seemed roughly split between preserving property rights and aiding the salmon.

Aside from habitat restoration, the only remaining way for humans to help coho is to raise them in captivity and release them into the wild. Unfortunately, conservation hatcheries have at best a mixed success rate, says John Carlos Garza, an NMFS geneticist in Santa Cruz. "Historically, our best guess is that hatcheries have overall had a detrimental effect on salmon populations," he says. One reason is inbreeding: When populations dwindle, the chances of breeding related individuals goes up, and the inbred offspring have poor survival rates in the wild, Garza explains. To mitigate this problem, Garza has helped the two coho hatcheries in central California implement a genetic matchmaking service. They now use DNA tests to select which pairs of fish to breed in order to maximize genetic diversity.

Garza also oversees an experimental project in which captive-bred fish are raised in fresh water and released into streams as adults. This offers advantages over the standard approach of releasing young fish born in the hatchery: For one, the offspring of the adult-released fish imprint on the stream where they hatched rather than on the hatchery. Garza thinks this approach may be valuable for repopulating streams where salmon have been completely wiped out.

These innovative methods may help make conservation hatcheries more successful, but they won't be enough to save the coho on their own, Garza and others say. "They're very expensive and intensive strategies that should be adopted only when there aren't a lot of other good strategies available," Garza says. Unfortunately, the central coast coho are running out of options. "Let's be clear," Garza says, "these are last-ditch efforts."

—GREG MILLER

ScienceNOW.org

## From Science's Online Daily News Site

### Colliding Particles Can Make Black Holes

You've heard the controversy. Particle physicists predict that the world's new highest-energy atom smasher, the Large Hadron Collider near Geneva, Switzerland, might create tiny black holes. Curiously, though, nobody had ever shown that Einstein's theory of general relativity predicts that a black hole can be made this way. Now a computer model shows conclusively for the first time that a particle collision really can make a black hole. <http://bit.ly/blackholes>

### Bats and Whales Share Sonar Protein

Bats and dolphins are about as different as mammals get. Yet, both home in on their prey by emitting sound waves and sensing the reflections, a process called echolocation. A new study shows that in both groups the same protein evolved in the same way to make that possible. Researchers say it's surprising to discover a molecular convergence in these very distantly related groups of animals. <http://bit.ly/batwhale>

### Cold War Split Birds, Too

The Cold War divided the people of Europe for nearly half a century, and it turns out humans weren't the only



ones stuck behind the Iron Curtain. Trade blockades led to vastly different numbers and types of invasive birds in Western and Eastern Europe, new research reveals. The findings, say experts, highlight the dramatic impact human activity can have on the success of alien species. <http://bit.ly/coldwarbirds>

### Birdlike Dinosaur Was Adept Glider

The fight over bird flight evolution is one of the longest-running and most heated debates in paleontology. Were the first flyers arboreal creatures that initially glided from treetops to the ground? Or were they bipedal ground runners with evolving wings that allowed them to take progressively longer jumps? The first flight tests of a foam model of a four-winged, feathered dinosaur lend credence to the former hypothesis. <http://bit.ly/dinoflight>

Read the full postings, comments, and more on [scienconow.sciencemag.org](http://scienconow.sciencemag.org).



## PHYSICS

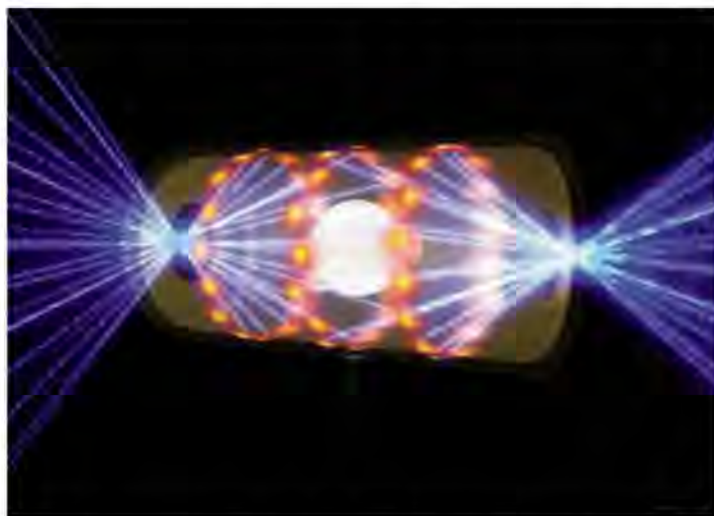
# Test Shots Show Laser-Fusion Experiment Is on Target

As the managers of the Large Hadron Collider at CERN near Geneva, Switzerland, can testify, building your giant research facility is one thing, but getting it to work properly when you switch it on is definitely another. Perhaps with CERN's setbacks in mind, those in charge of the National Ignition Facility (NIF), a huge laser for nuclear fusion experiments at Lawrence Livermore National Laboratory in California, have been gingerly putting their huge machine through its paces since it was completed nearly a year ago.

In a study published online this week by *Science* ([www.sciencemag.org/cgi/content/abstract/1185634](http://www.sciencemag.org/cgi/content/abstract/1185634)), NIF researchers describe their first experiments using all 192 of the facility's beams on test targets empty of fuel. They were able to couple the laser's energy into the target efficiently and implode the target symmetrically—so far, so good. The scientists say they are on target to attempt ignition—a self-sustaining fusion reaction that produces excess energy—before the end of this year. “It’s come up better than anyone thought. They’re ahead of the curve predicted,” says Mike Dunne, director of the Central Laser Facility of Rutherford Appleton Laboratory near Oxford, U.K.

NIF is the sledgehammer to crack a nut, writ huge (*Science*, 17 April 2009, p. 326). The sledgehammer is a laser that occupies a building the size of three football fields and 10 stories high. Inside it, hundreds of optical amplifiers, beam splitters, and other devices take a normal laser beam, split it 192 ways, and boost the combined energy of the beams to 1.8 megajoules.

The nut is a tiny spherical capsule the size of a peppercorn made of beryllium, which in later experiments will encase a dash of deuterium and tritium (D-T)—isotopes of hydrogen. The aim is to use the power of the laser to heat the capsule so fast that it explodes, propelling the D-T fuel inward toward the center and crushing it to a temperature and pressure greater than those in the core of the sun. As nuclei in the very center begin to fuse, the energy produced will cause all the D-T fuel to burn in a flash of energy—with luck, more energy than was pumped into the capsule in the first place.



**Hot cell.** To implode the central fuel capsule, NIF fires 192 beams into an eraser-sized gold cylinder, heating it to x-ray temperatures.

Livermore has spent more than 10 years and \$3.5 billion building NIF, and researchers hope successful ignition experiments will pay back that investment in future fusion power stations. But a main part of NIF's role is to test computer simulations of nuclear explosions to ensure that the U.S. nuclear weapons stockpile is reliable.

In the paper, Siegfried Glenzer of Livermore and his colleagues there, at Los Alamos National Laboratory, and at General Atomics in San Diego, California, describe shots using a beam energy of 0.7 megajoules, about 40% of NIF's maximum. “We’re doing the real thing, and it’s going better than expected,” Glenzer says.

The team addressed the problem of getting the most laser power onto the capsule and doing so symmetrically so that it implodes evenly. The laser's output is in the ultraviolet, but for a better implosion you need x-rays. So instead of shining the beams directly onto the capsule, they put it in the center of a gold cylinder about the size of a pencil eraser, called a hohlraum. By shining the beams through holes in the ends of the hohlraum, they can make its inner surface hot enough to emit x-rays, which cause the capsule to implode. In the experiments described, the team produced a radiation temperature inside the hohlraum of 3.3 million kelvin, exactly in line with models. Robert McCrory, director of the Laboratory for Laser Energetics (LLE) at the University of Rochester in New York state, calls the feat a major achievement. “If you don’t get the hohlraum temperature you’re not going to get the implosion you need.”

But the peril of this “indirect” approach is that gold atoms kicked off the inside wall of the hohlraum create a plasma that can interfere with the incoming beams in unpredictable ways. In the experiments, the NIF team managed to tune the many beams to keep these laser-plasma interactions to an acceptable level. “They were sufficiently benign at this energy, which is a huge success,” says Dunne.

In fact, the team turned some of these interactions to their advantage. In the past few years, theorists had suggested that with so many beams converging into the narrow ends of the hohlraum, the beams could nudge the plasma

into a regular repeating pattern, producing a sort of diffraction grating that might scatter the beams. It was potentially a “terminal problem,” says Dunne. But early last year, Livermore researchers suggested that they could use the gratings to steer the beams toward hard-to-reach parts of the hohlraum interior—in particular halfway down the inside wall, farthest from the entrance holes. The recent experiments have proved their theory right. “You can deposit energy where you need it,” Glenzer says. LLE's David Meyerhofer is impressed. “It’s the first time laser-plasma interactions have been used in a beneficial manner. Usually, you try to avoid them,” he says. “There’s no suggestion that this won’t work at full energy.”

The next major hurdle is ensuring that the implosion of the fuel is smooth and symmetrical. This remains “an open question,” Dunne says, because experiments so far have used empty capsules and the explosions “were not uniform on a microscopic scale, and that can cause problems later.” Even so, he says, he’s “not too worried yet,” because the capsules used were not machined to the same precision used for ignition shots.

Experiments at NIF are currently stopped, and the ignition campaign will begin in earnest in May, Glenzer says. If all goes well, Dunne says, a decision will be made in July on whether to push ahead with full D-T fusion experiments and an attempt at ignition in October. Successful ignition this year is “not out of the question,” McCrory says. “But I’d be surprised if it happens.”

—DANIEL CLERY

CREDIT: LANS/LNL/DOE



## PLANETARY SCIENCE

# Did a Battering Rain of Comets Bring Ganymede to Geologic Life?

Scientists have come up with a promising explanation of a planetary odd couple: Jupiter's major moons Ganymede and Callisto. Much like the disparate pair of Earth and Venus, the two moons have similar sizes and similar compositions but have followed vastly different paths of development. Once formed, Ganymede separated into layers of ice, rock, and molten metal, and then something reshaped parts of its surface. Meanwhile, Callisto went almost nowhere. It has not moved much beyond the bland, newborn ball of mixed ice and rock it started out as.

A study published this week in *Nature Geoscience* offers a possible explanation for why Ganymede evolved so much more than Callisto did: Ganymede suffered a far worse beating 3.9 billion years ago when a rain of comets and asteroids battered much of the solar system. "An interesting idea, well argued," says planetary physicist David

of the LHB's impacts on Ganymede and Callisto, they found plenty of discrimination. Jupiter's powerful gravity would have accelerated incoming comets and drawn more of them near the planet, the researchers note. Ganymede, being closer to Jupiter, would have suffered twice as many impacts as Callisto and at higher velocities. So Ganymede would have received 3.5 times more energy than Callisto.

In their modeling, that was more than enough heat to begin melting the ice in Ganymede's natal mixture of ice and rock. That thaw would have allowed the moon's rock to start sinking through the increasingly slushy interior. Then the sinking rock would have given up its gravitational energy as heat, accelerating the moon's separation into layers. Eventually, enough heat from radioactive decay would have built up to separate the rock's iron into a molten core, drive a magnetic field, and perhaps form Ganymede's grooved surface geology. Short-

changed on impact energy, Callisto would not have melted enough to achieve "runaway" heating during separation, leaving it cold and without a core. With a negligible heat source below it, Callisto's surface would be geologically dead for eons.

"It's a classic good-science paper," says planetary geologist James Head of Brown University. "It's very

exciting because it combines a couple of seemingly disparate problems." The scenario offers a promising, testable explanation for the Ganymede-Callisto dichotomy, he says. And to the extent that it proves to be an attractive explanation of the dichotomy, it also lends support to the reality of the LHB. In particular, Barr says, LHB heating "fits nicely with the Nice model." That model (named after the French city where it was developed) shows how Jupiter and Saturn could have stirred up an LHB while migrating outward through the solar system (*Science*, 17 July 2009, p. 262). But researchers agree that much analysis remains to be done, and another mission to the Jupiter system would be nice.

—RICHARD A. KERR



**No longer two peas in a pod.** Some source of heat—possibly a storm of impacts eons ago—led to the resurfacing of Ganymede (left) but left Callisto (right) largely unchanged.

Stevenson of the California Institute of Technology in Pasadena. "I think it's promising."

For 30 years, researchers have wondered what process could have got enough heat into Ganymede to drive its geological evolution without setting off Callisto as well. In search of a heat source that could discriminate between the two moons, planetary scientists Amy Barr and Robin Canup of the Southwest Research Institute in Boulder, Colorado, considered the late heavy bombardment (LHB). That's a hypothesized storm of comets and (in the inner solar system) asteroids that many planetary scientists think pummeled the solar system 3.9 billion years ago.

When Barr and Canup simulated the effects

## ScienceInsider

### From the Science Policy Blog



Writing in *Der Spiegel*, three prominent climate scientists have criticized the policies of the **Intergovernmental Panel on Climate Change** and its chair, **Rajendra Kumar Pachauri**. *The Wall Street Journal* reprinted the column, which says the panel should adopt conflict-of-interest policies, a mechanism for dealing with errors, and more transparent policies for selecting its leadership and authors. <http://bit.ly/7WX0ph>

Director Peter Sawicki has been let go by the **German Institute for Quality and Efficiency in Health Care**, and Fotis Kafatos is voluntarily stepping down from his position as president of the European Research Council. <http://bit.ly/5aNCJF>

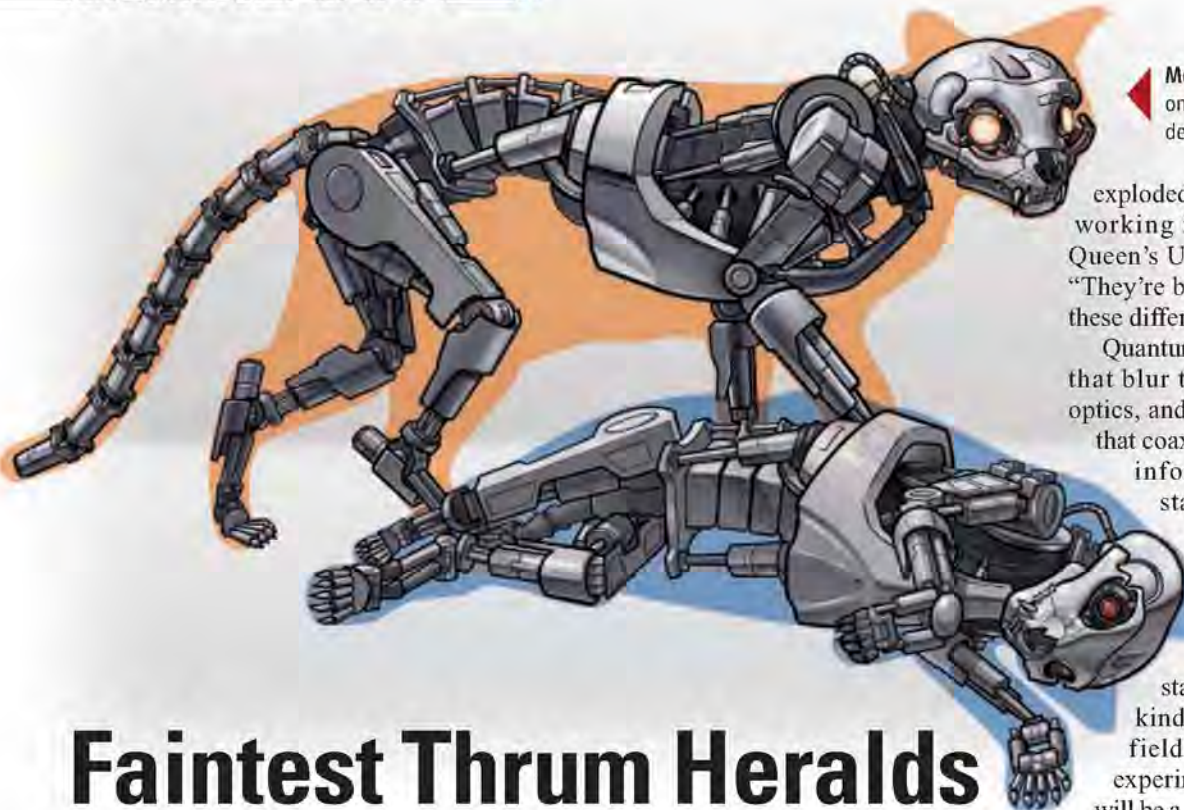
Budget woes have imperiled the dream of Alain Beaudet, president of the Canadian Institutes of Health Research, to launch a **massive initiative to combat Alzheimer's disease and dementia**. A government deficit has scaled back the proposal from hundreds of millions of dollars to a far more modest \$10 million. <http://bit.ly/7dRBWi>

A new report says that far fewer people than believed have died in the **Democratic Republic of the Congo** from a decade-long civil war. The International Rescue Committee has cited a widely quoted figure of 5.4 million, but the new analysis, part of a publication called the *Human Security Report 2009*, faults the committee's methods and suggests that the number is closer to 900,000. <http://bit.ly/4TJZAc>

A California physicist suggests that **delivering food to Haiti** by small, widely dispersed air drops would be more effective than delivering it to a few sites in large packages, which can be hard to distribute because of poor infrastructure or thieving thugs. Bill Wattenburg says the U.S. military has been wary of the technique, however, despite its successful use in Bosnia and Afghanistan. <http://bit.ly/6Xfg2O>

For the full postings and more, go to [blogs.sciencemag.org/scienceinsider](http://blogs.sciencemag.org/scienceinsider).





**Meow.** A gizmo occupying two places at once would be an analog to Schrödinger's dead-and-alive cat.

# Faintest Thrum Heralds Quantum Machines

**After years of trying, physicists are on the verge of making tiny vibrating devices that make the slightest possible movement. Far weirder mechanical devices could follow**

**IF YOU WANT TO BE REMEMBERED, MAKE A** bad prediction. In 2003, *Science* reported that within 6 months physicists might create tiny machines whose movement obeyed the weird rules of quantum mechanics, which state that an object can absorb energy only in discrete "quanta" and can be in two places at once (*Science*, 3 January 2003, p. 36). That didn't happen, but Tobias Kippenberg, an experimenter at the Swiss Federal Institute of Technology, Lausanne, still shows a slide of the article in his talks. "It's entertaining, and it also reminds people exactly how challenging the problems are in this field," Kippenberg says.

Those obstacles are not insurmountable, however. Kippenberg's team and several others have nearly entered the realm of quantum motion. Ironically, to get there, they're racing to make gizmos that make literally the slightest movement, vibrating widgets drained of every possible bit of energy and quivering with only an unquenchable "zero-point motion." Recent progress toward that "ground state" of motion has come so fast that even Kippenberg is willing to prognosticate: "I expect in the next year there will be

maybe a half-dozen groups observing this," he says.

If so, then the age of quantum machines will finally have arrived. Molecules, atoms, and subatomic particles all obey the mind-bending dictates of quantum theory. But physicists have never observed such odd behavior in the movement of a humanmade object. So their first goal has been to put the simplest machine—a vibrating beam or some other "oscillator"—into its ground state, which would be a crucial first step toward machines that oscillate around two different positions at once and other weird states of motion.

Within the past 6 months, four different groups have come within a few dozen quanta of that goal, and researchers say one may have reached it.

Curiously, as physicists have homed in on their objective, the field has grown more diverse. Seven years ago, a few groups of condensed matter physicists were pursuing a single strategy for reaching the ground state. Now, teams from optics and astrophysics have joined the chase. "The field has

exploded in terms of the number of people working in it," says Robert Knobel of Queen's University in Kingston, Canada. "They're bringing their toolboxes from all these different fields."

Quantum machines could lead to devices that blur the lines between electronics, optics, and mechanics—humming widgets that coax light into odd states or translate information encoded in quantum states of photons into electronic signals. They might even probe a fundamental mystery: Why don't human-scale objects behave quantum mechanically? Reaching the ground state of mechanical motion is "the kind of result that will create a new field in itself," says Jack Harris, an experimenter at Yale University. "This will be a door opening—a big one."

## High frequencies and low temperatures

But first physicists must make machines that make the slightest movement. Twentieth century physicist Werner Heisenberg's famous uncertainty principle implies that, like a small child, no object can stand perfectly still. Even in its ground state, it must possess a last, inextricable half-quantum of energy and jiggle with zero-point motion. Physicists would like to see that minimal tremor in a mechanical widget. To spot it, they are tinkering with nanometer-sized beams of semiconductor, micrometer-sized bits of glass, and even mirrors weighing kilograms.

Those objects all share a key property: When each is nudged away from its equilibrium position or shape, it oscillates at a well-

defined frequency like a tuning fork. Quantum mechanically, such a "harmonic oscillator" can absorb energy only in quanta whose size is proportional to its frequency. So to reach the ground state, physicists need to extract all

but the last, irretrievable half-quantum. That's easier said than done. Each quantum is so small that to remove them all, researchers must lower the oscillator's temperature—and hence its energy—nearly to absolute zero.

Nevertheless, physicists tried the direct route to the ground state. To make the energy quanta as large as possible, they etched beams of semiconductor that vibrated at high frequencies—up to 1 billion cycles per second, or 1 gigahertz. To make the beams

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**S** Podcast interview  
with author  
Adrian Cho.



as cold as possible, they relied on so-called passive cooling: sticking them in the best liquid-helium refrigerators, which can reach a few millikelvin, or thousandths of a degree above absolute zero. To sense a beam's motion, they employed a scheme called "capacitive coupling," applying a voltage between the beam and an electrode (see diagram). The beam's motion causes the voltage to vary.

Researchers ran into a few roadblocks, however. To push the frequencies of their vibrating beams higher, experimenters made them ever stiffer. But that meant that the size of the beam's already minuscule oscillations decreased even further, making them difficult to detect.

The scheme for measuring the beam's motion actually jostled the beam as well. Physicists tracked the varying voltage by observing how it affected the current through a device called a single-electron transistor. But as the electrons hopped through one by one, they tugged on the beam, creating "back action." "This back action is quite a bit bigger than you need for these quantum measurements," Knobel says. "And I don't think we understood that theoretically or experimentally at the time."

### Cool new ideas

Even as the straight path to the ground state proved difficult, new avenues toward that goal began to open. In particular, experts in quantum optics began experimenting with techniques to control the motion of micrometer-sized objects with laser light. "It turns out that you can use the whole quantum-optics toolbox to prepare, manipulate, and read out a mechanical system," says Markus Aspelmeyer, a physicist at the University of Vienna.

Ironically, physicists can cool an oscillator by shining light on it. Conceptually, such active cooling works as follows: Researchers put a tiny mirror on an oscillating beam (see diagram). It and a larger, fixed mirror form an "optical cavity" that resonates with light of a frequency set by the mirrors' spacing, just as an organ pipe whistles at a pitch set by its length. If the little mirror could not move, then only light of this frequency could shine through the large mirror and into the cavity.

But if the mirror oscillates at a definite frequency, then laser light whose frequency has been lowered by that amount can also enter the cavity. To do so, however, each photon must absorb a quantum of energy it is lacking. So that "detuned" light saps energy from the oscillator. The light wave

reemerging from the cavity also reveals the mirror's motion through shifts in the alignment of its peaks and troughs, or "phase," allowing researchers to detect the motion with greater sensitivity.

Using such "resolved-sideband cooling," three groups have reduced the energy of a micrometer-sized oscillator to between 32 and 67 quanta, as they reported in July 2009 in *Nature Physics*. The experiments varied in details. Aspelmeyer's team used a mirror on a beam; Kippenberg's shined light into a glass ring that served as both optical cavity and oscillator. Hailin Wang's team at the University of Oregon, Eugene, used a glass bead in a similar way. All three worked in fridges that reached a few kelvin; they think they can reach the ground state by starting at millikelvin temperatures. "It doesn't look like we are bumping into any fundamental issues" that prevent it, Aspelmeyer says.

Physicists working with nanometer-sized oscillators have found better ways to chill and measure their devices, too. In fact, they've borrowed the concept of resolved-sideband cooling. For example, Keith Schwab of the California Institute of Technology (Caltech) in Pasadena and colleagues have applied it to a silicon-nitride beam 30 micrometers long and about 150 nanometers thick and wide that thrums at 6.3 megahertz.

The beam couples to a nearby microwave resonator—simply a long strip of superconducting niobium that can ring with microwaves of a particular frequency. As in the optical experiments, detuned

microwaves can enter the niobium strip only by absorbing energy from the beam. Starting at millikelvin temperatures, the researchers reduced the beam's energy to a handful of quanta, as they reported this month in *Nature*. "We pushed the experiment as hard as we could, and in the end we came down to four," Schwab says.

### Brute force carries the day

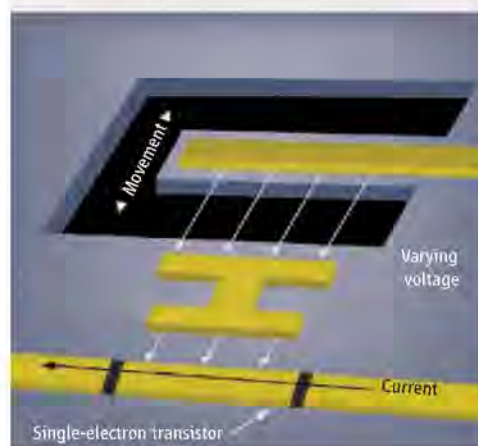
In the race to the ground state, however, the straightforward approach seems to have won out. Andrew Cleland, John Martinis, and colleagues at the University of California, Santa Barbara, have succeeded by using a "brute force" combination of passive cooling and a very high oscillator frequency, say several physicists who have seen preprints describing the work. The key to the experiment lies in a clever scheme to detect the oscillator's motion, they say.

Cleland and Martinis's gizmo is a beam that vibrates at a whopping 6 gigahertz, researchers say. But rather than swinging up and down, it gets thinner and thicker. It also consists of so-called piezoelectric material that creates an oscillating electric field as it expands and contracts, making that motion easy to detect.

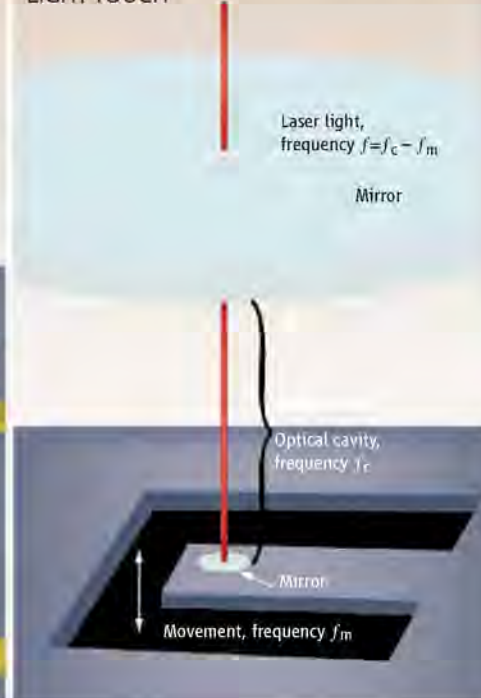
To do that, Cleland and Martinis rely on a widget called a "phase qubit," a strip of superconductor with a nonsuperconducting patch in it that acts a bit like a sandbar in a stream of free-flowing electrons. The details aside, the phase qubit is itself a highly controllable quantum-mechanical system with a

**Chillin'.** In the old-school approach (left), physicists simply cool a vibrating beam in a helium refrigerator. In a new tack, laser light saps a beam's energy as it enters an optical cavity with the beam at one end.

### BRUTE FORCE



### LIGHT TOUCH





ground state and one higher-energy state. Researchers can ease the qubit from one state to the other—or even put it into both states at once—by applying microwaves of a specific frequency. Moreover, they can change that frequency by adjusting the current flowing through the qubit.

So Cleland and Martinis can feed energy quanta into the oscillating beam one by one. They first put the qubit into its energetic state and then adjust the qubit's frequency to match that of the oscillator to shuffle the quantum of energy over. They can also run the process backward to pull quanta out of the oscillator. And the team has pulled out every last one to reach the ground state, others say. "I would say that Andrew and John have achieved it," Schwab says. "We've gotten damn close, but these guys are deep into it."

Cleland and Martinis had previously used a phase qubit to fiddle with a microwave cavity. They showed they could put the cavity into any delicate quantum state, including the ground state or one in which it contained two different numbers of microwave photons simultaneously, as they reported in May in *Nature*. Now, they have simply replaced the gigahertz microwave cavity with a gigahertz mechanical oscillator, others say.

Not everyone is convinced that the Santa Barbara team has reached the desired goal. The researchers detect not the motion of the beam but the electric field the material produces, Kippenberg argues: "It's not a purely mechanical oscillator." The experiment could be done with a different material that would produce such a field through internal stresses, without mechanical motion, he says. Others say that's a quibble, as the Santa Barbara device does move.

### Testing the limits of reality

Just what quantum machines will be good for remains to be seen. Most immediately, they might have technical applications in basic research. A gigahertz nanomechanical oscillator in its ground state would make an exquisitely sensitive force detector, says physicist Konrad Lehnert of JILA, a laboratory run jointly by the National Institute of Standards and Technology and the University of Colorado, Boulder. "That gives you a way to interrogate the nanoscale world in a very gentle, noninvasive way," he says.

Micrometer-sized widgets in quantum motion might prove particularly useful in quantum optics experiments, says Yale's Harris. They might serve as "nonlinear" optical elements that perform tasks such as splitting a single higher-energy photon into

two lower-energy ones, he says. Currently, researchers do such things by passing light through certain crystals, which typically absorb a large fraction of the photons. Quantum machines might do the job without such high losses, Harris says.

The tiny machines might also bridge the gaps between various technologies. Physicists can already control the quantum behavior of electrons and of electromagnetic waves such as light or microwaves. Quantum



**Many sizes.** Physicists are experimenting with nanometer-sized beams (top), micrometer-sized rings (middle), and macroscopic mirrors.

machines would enable them to control quantized vibrations, or "phonons," and forge connections among all three, says Oskar Painter, an applied physicist at Caltech. "You've got phonons, photons, and electrons" working together, he says. "That's where the revolution is going to come from."

Painter is already pushing in that direction. His team recently fashioned a beam of silicon 30 micrometers long and 1.4 micrometers wide and patterned it with a ladderlike arrangement of holes. The pattern simultaneously traps light and vibrations. In fact, the pressure from trapped light can set the beam

vibrating, and that motion reveals itself in microwaves emanating from the beam, the team reported in October 2009 in *Nature*. Such a structure could convert optical signals to microwaves and vice versa, Painter says.

Quantum machines might ultimately probe the origins of everyday reality. Although the rules of quantum mechanics allow an object to be in two places at once, human-sized "classical" objects do not behave that way. "Systems either behave quantum mechanically or classically," says Nergis Mavalvala, an astrophysicist at the Massachusetts Institute of Technology in Cambridge. "Is there something murky in between? I don't think anyone has an answer for that."

Many physicists think that in principle a large object could be put into such a two-places-at-once state—if it were shielded from vibrations, radiation, and other environmental influences, which cause such delicate states to "collapse." Others argue that as-yet-unknown factors may prevent large objects from behaving quantum mechanically. Famed British theorist Roger Penrose argues that if a large object were put into a here-and-there state, its own gravity would pull it to one place or the other, taking the quantum weirdness—and perhaps some of the fun—out of the everyday world.

To test such ideas, many researchers would like to try to put a human-sized object in two places at once. "There's nothing better than an experiment that proves that it works or it doesn't," Mavalvala says. Such experiments are a ways off, but physicists are surprisingly close to reaching the ground state of a jumbo oscillator. Using optical techniques, Mavalvala and colleagues cooled a 1-gram mirror to 100,000 quanta, as they reported in 2007 in *Physical Review Letters*.

More recently, the group has cooled mirrors weighing 10.8 kilograms even further. The four mirrors form two crossed 4-kilometer optical cavities in the Laser Interferometer Gravitational Wave Observatory (LIGO) in Hanford, Washington, which is designed to detect ripples in spacetime. Using LIGO's electronic stabilization system in lieu of lasers, the team cooled the mirrors' relative motion to just 234 quanta, as they reported in July 2009 in the *New Journal of Physics*.

Exactly where quantum machines will lead may be impossible to say. But once physicists can put gadgets into quantum motion, the possibilities may be limited only by researchers' ingenuity. Something new and wild seems sure to shake loose.

—ADRIAN CHO

CREDITS (TOP TO BOTTOM): TRISTAN ROCHELEAU ET AL., NATURE 463 (7 JANUARY 2010); T. J. KIPPENBERG AND G. ANETISBERGER/MAX PLANCK INSTITUTE OF QUANTUM OPTICS; COURTESY OF THE LIGO LABORATORY





## EVOLUTION

# In the Deep Blue Sea

A survey of corals and their kin shows the ocean's evolutionary potential

The deep sea may not seem like a crucible of evolution. But, to the surprise of biologists, a new construction of the coral family tree suggests that evolution proceeds at full bore in waters well below where sunlight penetrates. Moreover, some coral diversity may have bloomed there first, before spreading coastward—the reverse of what has long been thought. “As people look in the deep sea, they are finding much more diversity than they expected,” says Clifford Cunningham, an evolutionary biologist at Duke University in Durham, North Carolina. “We’re just at the very beginning of understanding deep-sea evolution.”

At a meeting\* in Seattle earlier this month, a dozen experts celebrated progress in assembling an improved family tree showing the relatedness of jellyfish, corals, anemones, and hydra, which are collectively known as cnidarians. Relatively simple creatures characterized by their production of specialized stinging cells, cnidarians are important to the marine ecosystem. Some live in deep water; others in the shallows. Some, like reef-forming corals, live in large colonies and partner with algae; others go it alone.

By comparing a variety of genes among cnidarians, Cunningham and the others gathered in Seattle have pieced together large swaths of their family tree. This Cnidarian Tree of Life project and other studies have unveiled where some of the species arose and where traits such as coloniality came from. The most paradigm-shaking result so far concerns certain corals. Researchers have long thought that deep-sea corals were derived from a few species of shallow-water reef dwellers that migrated out to sea over time. Those newcomers didn’t diversify very much in their new environs, it was thought.

But 40 years ago, marine biologists discovered quite a diversity of worms and other soft-bodied creatures in deep-sea sediments, with up to 100 species per shovelful of mud. Marveling over this surprising variety, Scott France of the University of Louisiana, Lafayette, wondered whether hard, rocky sea floors were also rich in coral species. Since 1992, he and his colleagues have been collecting specimens and data from submersible expeditions and from museum collections, focusing on black corals—named for their dark, flexible, treelike skeletons—and octocorals, all of which have eight tentacles as opposed to the black coral’s six.

To figure out who lived where, Eric Pante, one of his graduate students, charted the data on depths at which 3100 museum specimens were collected and incorporated specimens that he and France collected at sea. They found, for example, that most of 420 metallic-colored chrysogorgiid corals documented lived in the deep sea, but a few were confined to shallow water.

France and his colleagues also sequenced parts of several genes from representatives of three families of octocorals, those commonly known as sea fans and sea whips. Comparisons of the DNA sequences allowed them to make a family tree, upon which they could overlay the depths of each species’ home. The deep-sea species in the three octocoral families “all seem to be coming from a single common ancestor” that lived in deep water, France concluded at the meeting. Mercer Brugler, another of France’s graduate students, looked at three families of black corals, which are valued for jewelry, and concluded that they too diversified in the deep ocean.

This emerging story isn’t limited to corals. Marymegan Daly, a systematist at Ohio State University, Columbus, and co-coordinator of the Cnidarian Tree of Life project, has found a similar pattern among the sea anemones she’s analyzed. “We see lots of radiations of deep-

**Diversity in the dark.** (left to right) A colony of bamboo coral, a solitary deep-sea coral, and a chrysogorgiid octocoral speak to the richness of the ocean depths.

sea forms,” she reported. In short, concludes France, “we can’t say that the deep sea is a boring environment in terms of evolution.”

And although the Cnidarian Tree of Life project has confirmed that other types of corals have shallow origins, those lineages may find refuge in the deep sea during tough times, says Marcos Barbeitos, an evolutionary biologist at the University of Kansas (KU), Lawrence. Barbeitos studies solitary corals, which are individual polyps, and some of their reef-forming counterparts, which are large colonies of polyps that tend to live in shallow water. Taxonomists have tended to group reef corals with each other, putting deep-water solitary corals in a separate group. They have also assumed that the colonial corals arose from an ancestor that was a solitary coral. But Barbeitos’s study, based on DNA samples from 97 coral species, suggests that some solitary corals are closer kin to shallow-reef colonial species than to other deep-water solitary corals and that the evolutionary path to coloniality may equally well go the other way.

A statistical analysis revealed that at least two of the solitary lineages from the deep sea evolved from colonial forms rather than the other way around. These colonial forms apparently “diversify in shallow water and give rise to [solitary] species that live in deeper water,” says Barbeitos. Furthermore, his data indicate that colonial forms are about as likely to arise from solitary forms as the other way around, suggesting that coloniality can be lost and gained over evolutionary time.

Barbeitos suggests that as reefs waxed and waned over the planet’s history, corals have managed to survive in part because deep-water lineages persist. They then expand and diversify into shallow-water, reef-forming corals when conditions are again favorable. As with the black and octocorals, deep water is critical to Cnidarian evolution, says KU’s Paulyn Cartwright, co-coordinator of the Cnidarian Tree of Life project. Corals “may be more robust because of the connection to deep-water relatives.” —ELIZABETH PENNISI

\*The Society for Integrative and Comparative Biology meeting took place 3–7 January in Seattle, Washington.





## RESEARCH FACILITIES

## Little Castle on the Prairie

Can \$400 million and a "Manhattan Project for diabetes" transform South Dakota into a research hub?

The flagship building for Sanford Health in Sioux Falls, South Dakota, really does fly flags, colored pennants over ramparts and turrets. Shields with pseudoheraldic symbols hang inside, and wooden thrones overlook the lobby. This children's hospital runs on what it calls the "Disney principle": Just as children never see the Mickey Mouse mascot at Disneyland without his head on, Sanford never lets the fantasy lapse with a bare surface. CT scanners have twisting otters painted on them, and doors of tiny, Wonderland size appear in the hallway, opening onto dioramas of secret worlds. Had Alice fallen ill, she would have convalesced at something like the Sanford Castle.

With so much invested in decoration, the castle is decidedly a patients' hospital. Indeed, the focus of Sanford Health (a cluster of clinics formerly known as Sioux Valley Hospital and Health) has always been patient care, not clinical science. But recently, the institution's stepsister of a research arm was transformed. T. Denny Sanford, a banking mogul, had already donated \$16 million to build the castle in 2004, an astounding amount for South Dakota. To help push beyond caring for patients, in February 2007 Sanford donated \$400 million more, largely for medical research—one of the largest gifts ever bestowed on any U.S. hospital. Rich Adcock, an executive vice president at Sanford, said the hospital had a vision for growth, "but we knew we needed an angel. Denny Sanford was that angel."

With Sanford's blessing, officials announced what's known as the Sanford Project: a plan to cure one widespread disease within Sanford's lifetime. The deadline is a challenge; Sanford turns 75 this year. After interviewing experts, project directors selected type 1 diabetes (juvenile diabetes), a disorder in which immune cells destroy the body's beta cells, the insulin-secreting cells in the pancreas. Newt Gingrich, the former U.S. House of Representatives speaker and a health care-reform advocate, showed up to help announce what Sanford Health is calling a "Manhattan Project."

Conquering diabetes is ambitious enough, but then the project declared it had already settled on the approach it would take: harnessing the body's ability to regenerate beta cells. The choice startled some diabetes scientists who saw equal or more promise in other approaches—stem-cell therapy or beta-cell transplants. And the ultimate goal of the Sanford Project may be even more audacious: enticing enough scientific talent to create a permanent biomedical hub, a Boston or San Diego, in the 46th most populous state in the union.

### 'Let's start tomorrow'

Paul Burn, a biochemist and head of research for the Sanford Project, is exasperated that most clinical diabetes research focuses on preventing overactive immune cells from destroying beta cells. Doctors normally don't diagnose children with dia-

**Night lights.** Sanford Health plans to build about 20 children's clinics worldwide, all shaped like castles.

betes until they've lost 90% of their beta cells, so corralling their immune systems alone won't reverse the damage, he argues. He thinks it's essential to regenerate healthy beta cells to cure the disease.

But if Burn merely wanted to realign research priorities, he could have stayed at his old job as vice president of research for the Juvenile Diabetes Research Foundation (JDRF) in New York City. He came to Sioux Falls to run a different kind of clinic, one that, he says, "is making big bets." His team won't be doing basic research, though: "Our focus will really be on proof-of-concept studies," including clinical trials.

Burn sees a gap between research and drug development for type 1 diabetes and wants Sanford to bridge it. Based on the years he worked for drug companies, Burn argues that they have little financial incentive to run trials for type 1 diabetes when there's a larger and far more lucrative market for the distinct problem of adult-onset, or type 2, diabetes. (Burn estimates that 90% of research dollars spent on diabetes fund type 2 work.) "Even beyond 2015, there's no cure in the pipeline" for type 1, Burn says. "It's the same old story of trying to deliver insulin in a new way."

Because Sanford does little basic research, Burn hopes that places like the Diabetes Center of Excellence at the University of Florida, Gainesville, or the Burnham Institute based in San Diego (and the recipient of its own \$30 million gift from Sanford), will funnel promising lab results to him for clinical trials. Burn drew on his JDRF contacts to set up these partnerships, but the ties are loose, and no one will coordinate the direction of basic research among the clinics.

Burn is now interviewing for six positions to run clinical trials at Sanford, and he already has his core research team in place. The project impressed many doubters in June 2009 by unveiling a surprisingly international group, including scientists from China and Russia. (Burn, who is Swiss, has a Ph.D. from the University of Basel.) One early recruit was Alexander Rabinovitch, a gaunt Canadian forced into retirement because of his age by the University of Alberta in Edmonton. His lab in Canada had significantly boosted the numbers of beta cells in mice by using two drugs already approved by the Food and Drug Administration for other purposes, and he was eager to expand into human trials. No one seemed likely to sponsor the work, however. When Burn heard this, he immediately



dipped into the \$400 million and offered to fund it. It will start this March or April, the first trial initiated at Sanford.

It's an example of the flexibility that nine figures can buy. Sanford Health—which partners with the University of South Dakota School of Medicine—maintains that its annual research budget for diabetes will approach \$100 million per year. Burn hopes his scientists can win grants to cover much of that, but he can also cut through bureaucratic tape himself. “If they have a good project, I tell them, ‘Here’s the money, let’s start tomorrow.’”

Burn sees advantages even in the remoteness of Sioux Falls. With a dearth of competition from other research clinics, Sanford will have its pick of diabetes patients from the nearby population of 155,000. Sioux Falls does not support a deep scientific community like larger cities do, he admits, “but it’s a paradise from the point of view of trying to set up a clinical trial.” What’s more, Sanford Castle is just the first of 20-some castles planned. Another one financed by Sanford opened in August in Oklahoma, and administrators are scouting sites in Belize, Ireland, and elsewhere. Burn hopes to recruit diabetes patients through each castle.

### Creeping doubts

Not everyone imagines a research utopia in Sioux Falls. “I think some people really share my concern that it’s not an established scientific community,” says Gordon Weir, a Harvard University molecular biologist who works on regenerating beta cells.

Hendrik-Jan Schuurman, a diabetes researcher at the University of Minnesota, Twin Cities, is more supportive but echoes Weir’s concerns: “Everybody knows the university in South Dakota is not an institute with a reputation for biomedical research.” That’s why he thought Sanford was prudent to establish ties with Burnham and the Florida diabetes center, places

that can provide support and advice for Sanford while it builds up the long-term research infrastructure it will need.

Scientists have also questioned putting so much money into just one option—beta-cell regeneration—when other ideas seem worth trying. Schuurman and colleagues in New Zealand have developed ways to transplant pig pancreatic cells into patients, by coating the cells to enable them to escape detection by the immune system. Schuurman acknowledges



**Big bets.** Paul Burn wants to cure type 1 diabetes and build a research hub in Sioux Falls.

that pig cells are not a long-term solution—the donor pigs must be raised in supersterile bays at costs of \$50 per day per pig—but feels they are the best short-term hope (*Science*, 20 November 2009, p. 1049).

Weir touts regenerating beta cells from embryonic stem cells or induced pluripotent stem cells (iPS cells): adult cells that have been reprogrammed to a stemlike state. Adult cells present challenges, such as the need to remove epigenetic imprinting, Weir says, “and we’re still figuring out whether there are [other] differences that would not allow them to succeed.” For these reasons, he feels that generating beta cells from embryonic stem cells (ES) is the most attractive path.

Burn argues that stem-cell therapies, adult or embryonic, are 20 or 30 years distant, and he’s impatient. Yet there’s another reason Sanford will not sponsor embryonic stem cell work: South Dakota has banned it. A local advocacy group, South Dakotans for Lifesaving Cures, calls the ban the most

restrictive in the country, as it might prohibit citizens from receiving ES treatments developed elsewhere. The group plans to introduce a bill into the state legislature to lift the ban, partly to aid research, says spokesperson Nathan Peterson: “We have top-notch medical facilities, and there isn’t any reason the scientists shouldn’t have the option of conducting [ES] work in an ethical manner.” Sanford, however, refuses to take a public stand on lifting the ES ban.

### Room to grow

While the Sanford Project still publicly proclaims it will oversee \$100 million of research a year and cure diabetes through beta-cell regeneration alone, in private Burn backs off a bit. The project will spend about \$30 million in 2010, and he diplomatically calls \$100 million an “ambitious” number. He also says Sanford will explore areas beyond regeneration, perhaps dabbling in iPS or even immunological work.

At the same time, Burn isn’t shying away from the Sanford Project’s vow to cure type 1 diabetes, soon. He’s overseeing a move into a new headquarters, a 28,000-m<sup>2</sup> building on the outskirts of Sioux Falls. Burn hopes to move 150 people into one of its two 5,600-m<sup>2</sup> research bays by this summer and fill a second bay later. “At universities, you’re often fighting over every square foot,” Burn notes from a snowy and truly capacious parking lot. “Here space should be no issue.”

As for the future, Sanford Health plans to open a 75-hectare research campus south of town and keep the momentum of the Sanford Project going after it cures type 1 diabetes. Burn believes beta-cell regeneration holds great promise for curing type 2 as well. Indeed, Burn sees no reason Sanford can’t build a green oasis of research in an otherwise barren scientific state. And even skeptics of the project admit that \$400 million is a lot of green. “You can create a scientific environment in even Timbuktu,” says Harvard’s Weir. “If you make it big enough and get enough people there, you can succeed.”

—SAM KEAN



**Doubts.** Harvard’s Gordon Weir and others ask if the project can succeed.





## LETTERS

edited by Jennifer Sills

## Tracking the Source of Glacier Misinformation

A RECENT NEWS OF THE WEEK STORY ON HIMALAYAN GLACIERS ("NO SIGN YET OF HIMALAYAN meltdown, Indian report finds," P. Bagla, 13 November 2009, p. 924) highlights how inadequately reviewed material makes its way into the public consciousness. One source, Working Group II (WG-II) of the Intergovernmental Panel on Climate Change (IPCC) [pp. 493 and 494 in (1)] reproduces several errors. The Working Group writes that "[g]laciers in the Himalaya are receding faster than in any other part of the world" and that "the likelihood of them disappearing by the year 2035 and perhaps sooner is very high if the Earth keeps warm-

ing at the current rate. Its total area will likely shrink from the present 500,000 to 100,000 km<sup>2</sup> by the year 2035." Another source (2) advances a no-less mistaken conjecture, not discussed in Bagla's News of the Week story, that Himalayan glaciers are responding to the climate of as long as 15,000 years ago.

The IPCC Fourth Assessment, particularly of the physical science basis for the changes, is mostly accurate, but the first WG-II sentence above derives from a World Wildlife Fund report (3), which cites a news story (4) about an unpublished study (5) that neither compares Himalayan glaciers with other

rates of recession nor estimates a date for disappearance of Himalayan glaciers. Himalayan rates of recession in the WG-II report (1) are not exceptional (6). In the second WG-II sentence, "its" cannot refer to Himalayan glaciers [area about 33,000 km<sup>2</sup> (7)], and may refer to the world total area of glaciers and ice caps. A bibliographic search suggests that the second WG-II sentence is copied inaccurately from (8), in which the predicted date for shrinkage of the world total from 500,000 to 100,000 km<sup>2</sup> is 2350, not 2035.

The claim that Himalayan glaciers may disappear by 2035 requires a 25-fold greater loss rate from 1999 to 2035 than that estimated for 1960 to 1999 (7). It conflicts with knowledge of glacier-climate relationships and is wrong. Nevertheless, it has captured the global imagination and has been repeated in good faith often, including recently by the IPCC's chairman (9).

These errors could have been avoided had the norms of scientific publication, including peer review and concentration upon peer-reviewed work, been respected.

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## A Role for Postdocs in Undergraduate Education

IN HIS EDITORIAL, "GALVANIZING SCIENCE departments" (4 September 2009, p. 1181), C. Wieman described ongoing programs at University of Colorado, Boulder and University of British Columbia in Vancouver that are successfully implementing new effective, research-based teaching methods in several science, technology, engineering, and mathematics (STEM) departments. As Wieman points out, transformations in the STEM teaching culture at large research universities are sorely needed, but such institutional change is notoriously difficult to bring about. It is therefore worth a closer look at how these two programs work. Their success has been primarily due to the science education specialists Wieman mentions, who are called Science Teaching Fellows (STFs) in Boulder. It may not have been clear from the Editorial that these are postdocs. Most earned Ph.D.s in their respective science disciplines (not education), but developed strong interests in pedagogy and educational research during their training.

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One of their missions is to assist departmental faculty with the kinds of course transformation that Wieman describes. As post-docs in the discipline, they have the content knowledge required for effective development of educational materials, and they are not threatening to faculty, as outsiders with educational degrees might be. Their ability to effect faculty change derives from their familiarity with the educational research evidence, their enthusiasm and people skills, and the assistance they can offer in implementing new teaching approaches, which can be labor-intensive.

The other mission for STFs is to gain science education training, which is uncommon within science departments and is valuable in light of the growing number of college and university science departments desiring permanent Science Faculty with Education Specialties (SFES) (1, 2). These individuals are discipline-based science faculty who make scholarly work in science education part of the fabric of the science disciplines themselves. SFES are undertaking efforts in the three science education arenas of undergraduate science education, K-12 science education, and discipline-based science education research, as well as in basic science research (2), furthering the current push to improve STEM education at all levels.

The Boulder and Vancouver programs, unique to our knowledge, should be transferable to any institution that can provide strong, pedagogically informed leadership (preferably from within STEM departments) and financial support for STFs. Funding agencies and foundations could have a major impact on improving STEM education by supporting such postdoctoral positions, thereby enabling the replication of these programs at other universities and promoting the training of more SFES.

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## Taking a Cue from the Silver Screen

IN HIS EDITORIAL "PROMOTING SCIENTIFIC standards" (1 January, p. 12), B. Alberts notes that many scientific projects are carried out by large teams, which makes attributing author contributions a problem. The concept of authorship is derived from a literary tradition, but novels and poems are written by no more than one or two people. Accordingly, authorship presumes that everyone makes an equal contribution to the piece. The International Committee of Medical Journal Editors guidelines on authorship, also known as the Vancouver guidelines ([www.icmje.org](http://www.icmje.org)) explicitly state that every author has equal responsibility for all material in the paper. That the new *Science* policies described by Alberts do not follow the Vancouver guidelines suggests that we need a new model for assigning credit to scientific projects.

Films might provide a better model for assigning credit than literature. Movie productions, like large scientific projects, represent the collaborative efforts of large teams, often working semi-independently of each other. The credits spell out who did what—director, cinematographer, screenwriter, and so on. There is no pretense that everyone who contributed to the film is an author of the film.

Honorary authorships are often given to principal investigators who provide resources, but minimal scientific input. Such investigators are analogous to film producers, who often set up financing and handle administration. It is appropriate that this important

work receives due credit, but that credit should not imply involvement in the creative process. Such contributions would probably not be recognized if the film industry were using, as science still does, the blunt instrument of authorship.

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## Give the "Fair Sex" a Fair Shake

AS A LONGTIME READER OF *SCIENCE*, AND THE invited food speaker to the New York Academy of Science's series "Girls Night Out," I take exception to the idea that the choice of topics condescends to women ("Science for the fair sex," Random Samples, 18 December 2009, p. 1597). When I see the statement, "Guess girls are interested in science only if you can find a link to food, love, or makeup," I see the attitude—all too familiar to those of us whose work crosses into social science—that nothing but cell biology and genetics constitute real science. The statement suggests that work dealing with quotidian matters such as food, love, or even makeup cannot possibly be scientifically rigorous or interesting. I would argue instead that rigorous scientific thinking thoroughly informs my research on the influence of politics on agricultural production and consumption, particularly with respect to obesity and food safety. My lecture to the "girls" on 16 February will be much the same as the talks I give to mixed-gender audiences of researchers, university professors, health professionals, government officials, and business leaders. I am curious to know whether social scientists are as tired as I am of colleagues characterizing our work as insufficiently scientific to be taken seriously.

MARION NESTLE

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## Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web ([www.submit2science.org](http://www.submit2science.org)) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.



## NEUROSCIENCE

## Making Sense of Printed Symbols

Charles G. Gross

There is a swath of cortex in the human right temporal lobe that is activated by the sight of words and letters. Damage to this “letter box area” results in a specific inability to read. The central “paradox” that Stanislas Dehaene’s *Reading in the Brain* tries to resolve is how the brain, the result of millions of years of evolution, can have mechanisms for reading (specifically, a particular area for processing words) when writing evolved only about 5400 years ago.

Dehaene, a leading cognitive scientist at the Collège de France, has made substantial contributions to our understanding of numbers and of reading, largely through imaging

**Reading in the Brain**  
The Science and Evolution  
of a Human Invention

by Stanislas Dehaene

Viking, New York, 2009.  
400 pp. \$27.95, C\$35.  
ISBN 9780670021109.

selectivity over changes in stimulus size, contrast, and exact location within the central visual field; and often have large receptive fields (the area of the visual field that activates them). Furthermore, their selectivity for shape can be modified (indeed programmed)

by experience, and their destruction results in a severe deficit in visual pattern recognition. Although studied in most detail in macaque monkeys, neurons with similar properties exist in the human temporal lobe as well. Thus these neurons are generally believed to underlie all types of visual pattern recognition even though they presumably evolved to recognize such things as the facial expressions of other monkeys, food, predators, and other visual patterns in the natural world.

Dehaene seems unaware of how plastic the properties of inferior temporal neurons are. For example, in monkeys, they can be taught to distinguish pet dogs from domestic cats (3), which hardly seems the result of natural selection. Similarly in imaging studies, there are regions in the temporal lobe of human car experts that respond differentially to different models of cars (4), again hardly the result of evolution. There is no paradox (or recycling) here: Inferior temporal neurons are general-purpose analyzers of visual forms—including cats, cars, words, and letters—that are relevant to the individual

because of previous experience.

Although the author’s treatment of behavioral and imaging studies of reading is quite sophisticated, his presentation of the supposed underlying physiology is often oversimplified and sometimes misleading. For example, he claims that many inferior temporal neurons code for shapes (“such as T, F, Y, or O”). In fact, neurons specifically responsive for these shapes over changes in size, contrast, and location have never been reported. Dehaene notes that “[m]ore complex curves, shapes, fragments of objects, or even entire objects or faces are, however, needed to trigger neurons at the higher levels” of the brain such as inferior temporal cortex. But the only objects that

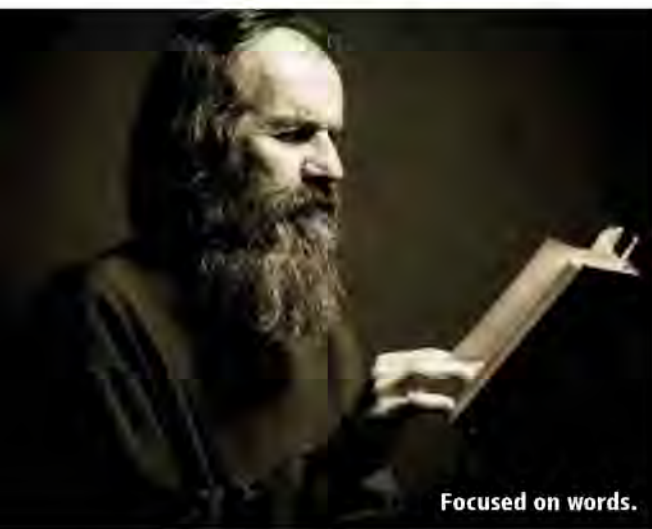
have ever been reported to be coded by single neurons (i.e., with responses invariant across changes in size, contrast, and location) are faces and hands (2). The hundreds of thousands of inferior temporal neurons studied by many groups of investigators provide no good evidence for a neuron coding any other object or any letter with such invariance. Even specific faces are not coded by a specific dedicated neuron or “grandmother cell”—a term, incidentally, introduced by Jerry Lettvin, not Horace Barlow (5). Dehaene seems to misunderstand the nature of visual receptive fields. Although the average size of receptive fields increases as one moves down the ventral visual system, this in no way implies “that the part of the retina to which the preferred object must be presented for the neuron to fire doubles or triples in diameter at each step.”

These errors suggest that Dehaene does not realize that individual faces and other objects are coded by the pattern of firing over a small population of neurons rather than by individual grandmother cells (5). For example, a given inferior temporal neuron may respond to four different faces, another neuron to two of these and to a fifth face, a third neuron to a different set of faces, and so on. Thus, each specific face is coded by the pattern of activity over a set of neurons rather than by the firing of a single dedicated neuron or a set of dedicated neurons with the same properties (4). Such population coding is presumably also the way objects and letters are coded.

The book ends with a (Faustian?) incantation to “the blooming of ... a ‘culture of neurons’” that will extend neuronal recycling to aspects of human culture beyond reading:

I would like to defend the idea that reading is only one of many examples of how cultural invention is constrained by our neuronal architecture. If we extend the neuronal recycling model to other human activities, we should be able to connect them to their corresponding brain mechanisms.... [W]e may one day be able to list the essential ingredients of all human culture (including family, society, religion, music, art, and so on) and understand how each of them relates to the vast array of our brain capabilities.

Something similar to Dehaene’s reading area paradox led Alfred Russel Wallace to a major break with Charles Darwin over the evolution of human cognitive abilities. On the basis of the years Wallace spent among Pacific Islanders, he believed them in no way intellectually inferior to Englishmen. Yet he thought their cognitive abilities in such areas



Focused on words.

studies in humans. In the book, he discusses how we read, how we learn to read, and how reading can be disordered. His account is critical, cautious, reasonably comprehensive, and accessible to laymen.

The author identifies the answer to his word-area paradox as the properties of neurons in the inferior temporal cortex, which he describes as having been recycled for reading letters and words. These visual neurons have been studied in monkeys for over 40 years (1, 2). They are selectively responsive to complex shapes; frequently maintain their

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as mathematics and music could not have arisen by natural selection because these abilities were not adaptive for “savages.” He resolved this paradox by attributing the evolution of human cognition to some spiritual force rather than natural selection (6), a view that caused Darwin to groan (7).

Some neuroscientists may groan over the presentation of familiar ideas in *Reading in the Brain* as if they have been newly discovered by the author—for instance, the ideas that brain plasticity, while considerable, is constrained by genetics; that neurons that evolved for one function can take on another through experience (Dehaene’s “neuron recycling”); and that humans can teach themselves far beyond the capacity of other animals. Alas, even the best popular science writing often leads experts to complain that what is well known to them is misleadingly presented as fresh and original. Nonetheless, for many readers those details may really be new.

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#### THEATER

## Of Locusts and Scientists

Manfred D. Laubichler and Gitta Honegger

Throughout history, the arrival of swarms of migratory locusts has brought famine, strife, and the dissolution of order. Even today, despite intensive worldwide monitoring under the auspices of the United Nations’ Food and Agriculture Organization, locusts continue to wreak havoc by destroying thousands of acres of farmland in such places as Somalia, which are at the brink of starvation even without the devastat-

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## BROWSINGS

### Strange Maps: An Atlas of Cartographic Curiosities.

Frank Jacobs. Viking Studio, New York, 2009. 256 pp. Paper, \$30, C\$37.50. ISBN 9780142005255.

This “anti-atlas” grew out of the blog (<http://strangemaps.wordpress.com>) that Jacobs started “to collect cartographic curiosia” from the Internet. It opens with a few examples of geographers’ misconceptions, such as Mercator’s late-16th-century *Septentrionalium Terrarum*, which placed a black and very high cliff at the center of a whirlpool around the North Pole. Several chapters present out-of-the-ordinary geographies: unrealized political proposals, ephemeral states, odd borders, and “bits of national territory adrift from the motherland.” Jacobs chose some examples for their unusual approaches, such as distinctive perspectives, transformations into living creatures, or distortions of propaganda and parody. He offers cartograms (“statistics masquerading as maps”) and other depictions of linguistic, culinary, cultural, and economic differences. There are literary creations (such as Captain Nemo’s Mysterious Island and Dorothy’s Land of Oz), as well as images formed by jam (the Americas) or clouds (Great Britain) or from beef (Brazil). Jacobs even includes a few extraterrestrial examples, such as the U.S. Geological Survey’s striking representation of the effects of meteorite impacts in *The Central Far Side of the Moon* (above). The author provides background details and often whimsical commentary for each of the more than 100 maps.



ing effects of locust swarms. It is thus highly surprising, to say the least, to find roughly 10,000 African migratory locusts (*Locusta migratoria*) in a 60-m<sup>2</sup> terrarium at the center of a theater production entitled simply *Heuschrecken* [Locusts].

The production could be seen at the Zürich Schauspielhaus’s new performance space Schiffbau this past fall, and this coming spring it will appear at the Hebbel am Ufer Theater in Berlin. Responsible for this unusual event is Stefan Kaegi, cofounder of the innovative and award-winning German-Swiss theater collective Rimini Protokoll. Kaegi and his partners Helgard Haug and Daniel Wetzel have developed a unique theatrical approach to both highlighting and interweaving diverse interests and objectives in exploring the “big questions” and a variety of contemporary issues and concerns. Casting “real” people—whom they call the “experts of everyday life”—rather than professional actors, the team draws from their protagonists’ diverse fields of specialization, expertise, and life experiences to illuminate a given topic from a variety of perspectives. They have based previous projects on a reexamination of *Das Kapital* in a global economy (*Karl Marx, Das Kapital, Erster Band*), the problems of identity and adoption (*Welcome to You*), physics at CERN and the quest for the Higgs parti-

cle (*Physik*), and the impending change in Cairo from live muezzins to recorded calls for prayer (*Radio Muezzin*).

In *Heuschrecken*, the plot unfolds following the standardized routine of a laboratory notebook that details observations and measurements related to the events in the terrarium (referred to as “the system”). An actress (Lara Körte) reads the entries from a detached position above the system and the audience. She first accounts for time and place

and some basic parameters (such as temperature, humidity, number of locusts, and number of audience members) before introducing the experts. These are Zakaria Farah, Somali-born and Swiss-trained professor emeritus of food chemistry at the ETH Zürich; Jörg Samietz, an expert in agricultural entomology and

the ecology of locusts; Barbara Burtcher, a physics teacher and financial adviser; the musician Bo Wiget; and the video artist Andi A. Müller.

For much of the performance, the terrarium is a closed system monitored by the observing experts from the outside. Occasionally, someone ventures inside (usually the entomologist and, toward the end of the two-hour production, the physicist in a space suit). The audience, seated on risers along two sides of the terrarium, watches the activities inside the system both directly and through video

#### Heuschrecken [Locusts]

by Stefan Kaegi;  
scenography by Dominic Huber;  
music by Bo Wiget

Rimini Protokoll. Schauspielhaus  
Zürich, October 2009. HAU Berlin,  
28 March to 10 April 2010. [www.rimini-protokoll.de/website/en/project\\_4200.html](http://www.rimini-protokoll.de/website/en/project_4200.html)



projections fed by several cameras directed by the experts placed at the short sides.

As the performance unfolds, the audience, guided by the experts, observes life and death inside the system—including mating, instances of cannibalism, and the striking speed with which freshly supplied green plants are devoured. In addition, the performance contains experiments that test various hypotheses, such as how changing the temperature in different parts of the system affects the location of locusts. There is also the unexpected, in the form of “locust music,” when induced movements lead insects to land on different sections of strings coupled with an amplifier. For all of these experiments and observations, the experts provide explanations of locust biology and behavior.

But *Heuschrecken* is much more than a docudrama illustrating locust biology. Like all Rimini Protokoll productions, it creates its own laboratory setting by concurrently staging multiple interwoven narratives based on the life stories of the experts and their relations to the events inside the system. The main character in this production is Farah, who connects his childhood experiences with swarms of migratory locusts in Somalia and Kenya with his subsequent career as food chemist and agricultural expert in different parts of the developing world and the problems he encountered along the way. Burtcher—whose perspective as a physicist and financial adviser brings together concerns about resource management and commodity prices, complex dynamics, and, finally, the fragility of the global ecosystem and humanity’s precarious place in it—provides another



**Making “locust music.”** The performance includes sounds produced by insects landing on different sections of strings connected to an amplifier.

thread. Entering the system in a space suit, she suggests simultaneously what the future of our planet might look like (the audience had previously been told how much more robust locusts are than humans) and what other worlds we might encounter at some future time may resemble (with free associations to both science and science fiction).

So what has all of this to do with theater? The theater is, by definition, a place for observation. Its stories are about sex, conflict, and death. It is driven by curiosity leading to discovery. Its very setting is thus experimental, suggesting a common root of science and theater, which was recognized by Aristotle, elaborated by Renaissance artists and scholars, and largely forgotten in more recent times.

The production of *Heuschrecken* brings back this deep connection between science and theater. Its main focus is on observation: The audience observes (along with the experts) the life of the locusts in the system. It also observes the experts observing nature and connecting their observations to their life stories. And lastly, the audience observes itself observing the multiple dimensions of the production. These multiple layers of observation and reflection are at the core of both the scientific and theatrical experience.

This experience is simultaneously entertaining and insightful. The unfolding stories have all the elements of good drama. However, this theater production is not simply about science. It stages the drama of science, as the spectator is kept in suspense about events in nature and in the life of the experts and becomes a participant in a scientific and theatrical experiment. The science and theater each illuminate the other without sacrificing scientific accuracy or theatrical imagination.

*Heuschrecken* thus differs remarkably from many recent attempts to bridge the gap between the “two cultures” of the sciences and the humanities and arts. Countless symposia have explored “the arts and science,” and the Sloan Foundation has spent considerable resources to bring “science” to the main stage. However, many of these efforts have been less about understanding science and more a sequence of clichés about the scientist as tragically mad genius (*Proof*), mischievous genius (*QED*), or misunderstood and neglected genius (*Arcadia*). By risking a different, experimental approach, Rimini Protokoll has succeeded in bringing science to life on stage.



**The scientist as explorer of new worlds.**

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CREDIT: (TOP TO BOTTOM) TONY SUTER/T+T FOTOGRAFIE, WWW.TTFOTO.CH



## CLIMATE CHANGE

# The Politics of Geoengineering

Jason J. Blackstock<sup>1,2\*</sup> and Jane C. S. Long<sup>3</sup>

Despite mounting evidence that severe climate change could emerge rapidly, the global reduction of carbon emissions remains alarmingly elusive (1, 2). As a result, concerned scientists are now asking whether geoengineering—the intentional, large-scale alteration of the climate system—might be able to limit climate change impacts. Recent prominent reviews have emphasized that such schemes are fraught with uncertainties and potential negative effects and, thus, cannot be a substitute for comprehensive mitigation (3, 4). But as unabated climate change could itself prove extremely risky, these reviews also recommend expanding geoengineering research. As such research is considered (5–7), a process for ensuring global transparency and cooperation is needed.

Geoengineering schemes can be divided into two very different categories: carbon dioxide removal (CDR) and solar radiation management (SRM) (4). CDR schemes such as direct air capture (8) or ocean fertilization (9) would remove the cause of climate change. However, technical challenges and large uncertainties surrounding large-scale CDR deployment, along with long delays in the climatic response to carbon forcing, mean that it would take decades to have notable effect (4).

Conversely, SRM could substantially influence the climate in months, but with much greater uncertainty about the net effects. SRM schemes such as stratospheric aerosols and cloud brightening aim to cool the planet by reflecting a fraction of the incoming sunlight away from Earth. “Natural experiments” caused by volcanoes have demonstrated the rapid impact potential of SRM, and such schemes should be technically simple to deploy at low cost, relative to mitigation (3, 4). But SRM would at best unevenly ameliorate regional climatic change and may have serious unintended consequences. For example, SRM could produce droughts and delay the recovery of the ozone layer by decades, while doing almost nothing to address ocean acidification. SRM is thus not a substitute for mitigation (3, 4).

Despite the limitations and risks, banning responsible SRM research would be a mistake. The ability to rapidly influence the climate

means SRM might be the only recourse should a climate crisis materialize. Moreover, the rapid impact, simple deployment, and low cost of SRM schemes make unilateral deployment a very real concern (10). More knowledge will help us craft good international governance and avoid rash unilateral actions.

Until recently, SRM research was largely politically benign, as it consisted only of model studies published in the open literature (3). In contrast, emerging laboratory-based development of SRM technologies raises the prospect that national or corporate interests might try (or appear to try) to control or profit from these schemes. Such a perception would be particularly likely if SRM research were framed in terms of national security, especially if the results were classified (11).

Field tests of such technologies can exacerbate these issues. Subscale field experiments designed to have demonstrably negligible environmental and transboundary impacts, such as those recently conducted in Russia (12), can be valuable for testing technologies and identifying the environmental risks of scaling up. But the controversy surrounding a 2009 Indo-German ocean fertilization experiment highlights both the difficulty of proving that the risks of any geoengineering field test are in fact demonstrably negligible, and the political sensitivities such tests can evoke (13).

As such, nations must carefully consider the signals that any unilateral field test sends to the international community. If conducted without international approval, a test perceived (even just politically) to present transboundary risks could spark international tensions, creating a global “crisis of legitimacy” (10). For subscale testing, we need politically acceptable scientific standards and oversight mechanisms for ensuring demonstrably negligible impacts.

Eventually, confirming the effectiveness of an SRM scheme would require large-scale tests with demonstrable climatic impacts—essentially low-level deployment. But because of the complexity and variability of the climate, it will be extremely difficult to attribute impacts and unintended consequences to any test (3, 14). This means liability for damages, real or perceived, would become a political challenge. For example, if the Asian or African monsoon were to weaken in a year following an SRM test—at the edge of natural variability, but still causing droughts and food shortages—uncertainty about causation could fuel accusations of responsibility.

Nations commencing geoengineering research must commit to full international collaboration and transparency.

As a first step to addressing some of these issues, scientists should propose international norms and best practices for research (10). The upcoming Asilomar conference on Climate Intervention Technologies in March 2010 will bring together ~150 scientists to begin this process. However, although necessary, such norms are not sufficient.

Issues of acceptable risk for subscale testing; if, when, and where climatic impacts testing should begin; or how SRM technologies should be managed are not just scientific. Such questions require a broadly accessible, transparent, and international political process. Vulnerable developing countries so far absent from SRM discussions must be engaged, and all stakeholders need to consider whether existing frameworks can facilitate this process, or whether new forums, treaties, and organizations are required.

Emerging national research programs—and even individual scientists—must forswear climatic impacts testing and carefully restrict subscale field-testing until approved by a broad, legitimate international process. All SRM research should be in the public domain and should be integrated into any subsequent international research framework. Programs should include international collaboration, communicate with developing nations, and prioritize research that has global versus national benefits. These steps will limit the new problems geoengineering research heaps on an already strained global climate agenda, preserving options for future international cooperation.

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# Arsonists in Rheumatoid Arthritis

Guy A. Zimmerman and Andrew S. Weyrich

**P**latelets are small anucleate cells that circulate in the blood of mammals and are critical effectors of hemostasis, blood clotting, and wound repair. Rheumatoid arthritis is a chronic inflammatory disease of humans that involves the innate and adaptive limbs of the host immune system, and is thought to be autoimmune in nature. Traditionally, platelets and rheumatoid arthritis don't go together. On page 580 of this issue, Boilard *et al.* (1) report that they do—microparticles released by platelets may be incendiary devices in the conflagration of a hot, swollen, and painful rheumatoid joint.

Although rheumatoid arthritis affects several tissues, destructive joint inflammation is a central feature (see the figure). The normal knee is a synovial joint that encloses a space containing a clear, viscous, largely acellular fluid filtrate of plasma and is bordered by synovium, a tissue consisting of lining cells, stromal matrix molecules, and blood vessels. Boilard *et al.* have discovered that platelet microparticles—vesicles shed by activated platelets (2)—are present in knee joint fluid in

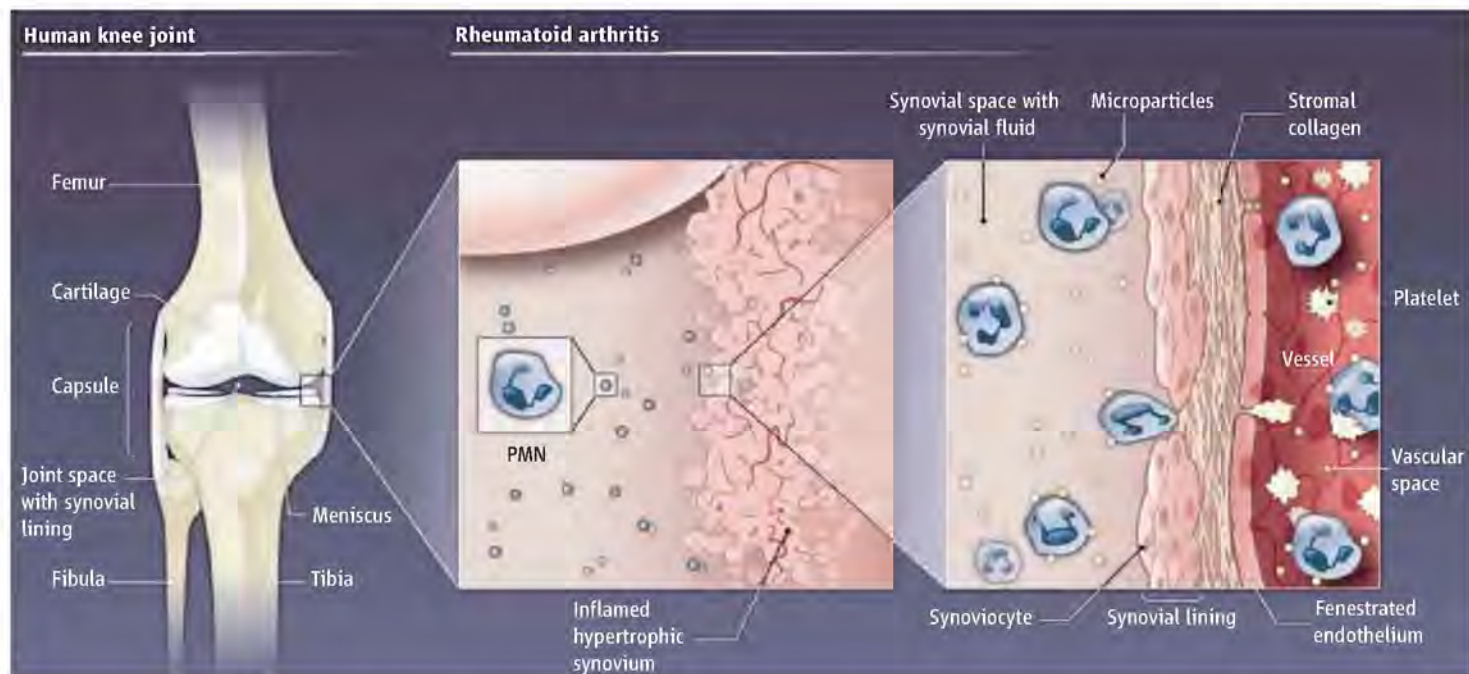
rheumatoid arthritis and other inflammatory arthritic conditions. The authors show, using a mouse model, that platelets are critical in the development of inflammatory arthritis. Activation of glycoprotein VI, a platelet-specific receptor for collagen, induces microparticle shedding. In addition, fibroblast-like cells that line the synovial cavity of the joint can also trigger microparticle release. Because these fibroblast-like synoviocytes and collagen are present in the inflamed synovium, platelet interactions in this milieu could lead to local release of microparticles and their translocation into the joint space.

Boilard *et al.* also determined that microparticles from the joint fluid of patients with rheumatoid arthritis can reciprocally activate fibroblast-like synoviocytes, and this interaction induces synoviocytes to secrete inflammatory chemokines and cytokines. Interleukin-1—a pleiotropic cytokine that is rapidly synthesized by activated human platelets (3) and is packaged into microparticles (1)—accounted for much of this stimulatory activity. Thus, a vicious cycle ensues: Fibroblast-like synoviocytes induce formation of platelet-derived microparticles. The microparticles then deliver interleukin-1,

Inflammation associated with rheumatoid arthritis is stoked by platelets, expanding their roles into the immune system.

which triggers synoviocytes to synthesize other cytokines and chemokines, some of which attract polymorphonuclear leukocytes and thereby fan the fire of inflammation.

A role for platelets has previously been described in inflammatory joint diseases, yet this is not commonly mentioned in summaries of their pathogenesis (4, 5). It's worth noting that platelets are previous suspects in rheumatoid arthritis: They accumulate in the joints of affected patients; platelet thrombi were observed in synovial vessels of patients with rheumatoid arthritis; and increased numbers of platelets in synovial fluid and of microparticles in blood are associated with the condition (6, 7–9). Why the lack of mechanistic study? Platelets have generally been viewed as cells with limited short-term hemostatic activities that exclusively take place in the vascular compartment. Recently discovered “new biology” of platelets is changing these perceptions, however. For example, they have an intricate transcriptome (entire repertoire of RNAs produced), activation-dependent posttranscriptional pathways, influences on extravascular events, and, perhaps, longer life spans than previously appreciated (10). Furthermore, there is a wealth of information



**Perpetrators in a painful joint.** In rheumatoid arthritis, the joint synovium becomes inflamed with dilated blood vessels and immune cells. Polymorphonuclear leukocytes (PMN), cytokines, chemokines, and other mediators of inflammation, accumulate in the synovial fluid. Local activation of platelets by collagen

and synoviocytes may trigger the release of microparticles from platelets. These microparticles then enter the joint space and further amplify inflammation by producing interleukin-1. This then activates synoviocytes, thus supporting a cycle of inflammation.

CREDIT: Y. GREENMAN/SCIENCE



indicating that platelets have inflammatory functions and a substantial arsenal of factors that confer the ability to signal to monocytes, dendritic cells, and other immune effector cells. There is also evidence that platelet microparticles activate adaptive immune cells in specific tissue compartments in response to cues that trigger antibody synthesis and alter lymphocyte activities (11).

A perplexing issue is that Boilard *et al.* did not detect intact platelets in the synovial fluid of patients with rheumatoid arthritis, although other investigators have found them (7, 8). How, then, did the incendiary microparticles gain access to the joint space? One possibility is that microparticles associate with transmigrating leukocytes and are then carried into the synovial fluid, a mechanism supported by microscopic and flow cytometric observations of Boilard *et al.*

This could happen in synovial blood vessels, where the numbers of microparticles may be much higher than reported in peripheral blood (9). It could also occur in extravascular synovial tissue if release of microparticles occurs as a result of platelet interactions with collagen and/or fibroblast-like synoviocytes in this compartment (1). Platelets adhere to activated polymorphonuclear leukocytes and monocytes in the circulation in many inflammatory conditions (12), including rheumatoid arthritis (13). Thus, leukocytes may deliver platelets and/or microparticles to extravascular sites by this, or other, mechanisms (14). Polymorphonuclear leukocytes may well be accomplices that deliver microparticles to the rheumatoid arthritis joint space and, conceivably, facilitate their local formation in transit through the synovium.

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## MATERIALS SCIENCE

# Bundling with X-rays

Cyrus R. Safinya<sup>1</sup> and Youli Li<sup>2</sup>

More than a century after its discovery, x-ray irradiation continues to profoundly impact a wide range of fields, from screening at airports to whole-body imaging diagnostics in health care (1). In the natural sciences, x-ray crystallography has clarified how the shapes of proteins and related complexes relate to their cellular function, and x-ray scattering has elucidated the structure and dynamics, mechanical properties, and intermolecular interactions of countless materials (2–5). On page 555 of this issue, Cui *et al.* (6) report a new twist in the application of x-ray scattering, where synchrotron x-ray irradiation, in addition to its usual role in probing structure, acts as a reversible switch for self-assembly from a disordered to an ordered state of bundled filaments (see the figure).

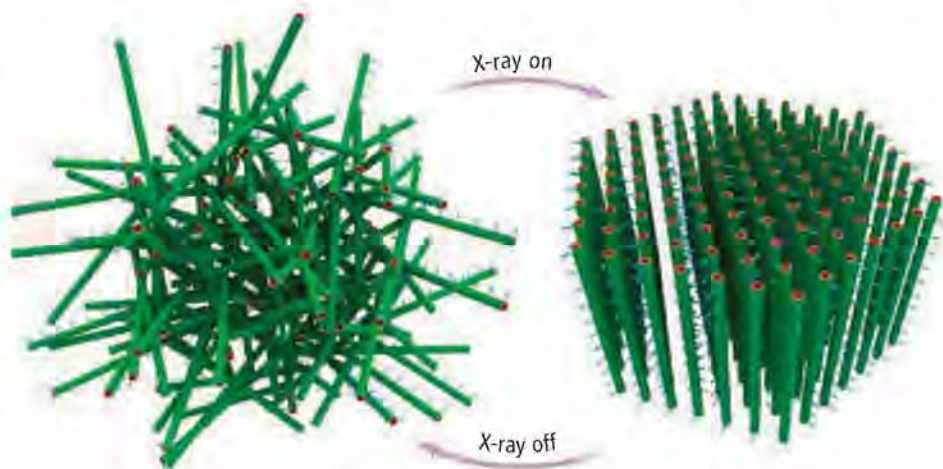
Bundling of filamentous proteins is widely observed in the eukaryotic cell cytoskeleton (7). In cells, bundles are assembled and disassembled to enable a wide range of functions. For example, filamentous actin can assemble with associating proteins into stress fibers ending in focal adhesion spots associated with cell adhesion, or form dynamically

active bundles within membrane-protruding filopodia in cell crawling. Another example is microtubule bundles, which are important in the development and extension of axons in neurons (8). The bundles are stabilized by microtubule-associated proteins and possibly other factors, which noncovalently cross-link neighboring microtubules.

Oosawa was the first theorist to develop a model explaining how nanofibers carrying the same charge may form bundles (9).

High-brilliance synchrotron radiation is used to trigger a reversible transition from randomly oriented filaments to hexagonal bundles.

The model, which applies generally to both biological and nonbiological fibers, is counterintuitive, because similarly charged fibers normally repel each other due to electrostatic forces. Oosawa showed how fluctuations in the bound counterions of neighboring filaments become correlated and produce an attractive force (similar to an effective van der Waals attraction), which leads to bundles. More recent theories also show the possibility of bundle formation through salt-bridge–



**X-ray irradiation switch.** In Cui *et al.*'s experiment, transmission of a high-brilliance synchrotron beam through the sample charges up peptide-based nanorods. As a result, the randomly oriented filaments transition to two-dimensional ionic crystals of hexagonal bundles. The filament bundles revert to the disordered state after the beam is turned off.

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like, exponentially decaying attractive forces between neighboring filaments (9).

Most experimental data on nonspecific bundling interactions appear to be consistent with theoretical predictions of densely packed bundles resulting from counterion-induced attractive forces, but substantial discrepancies remain. Microtubules can form various bundling architectures, from tight hexagonal bundles to loose two-dimensional necklace-like morphologies with linear, branched, and loop morphologies (10) that are not predicted by theory.

In contrast to the filament bundling described so far, which typically arises from attractive forces, long-range electrostatic repulsion appears to play the dominant role in inducing the formation of widely spaced, stable hexagonal filament bundles reported by Cui *et al.* The authors hypothesize that x-ray irradiation induces a reversible chemical reaction, with deprotonation of carboxyl groups on glutamic acid residues leading to highly charged filaments (see the figure). Their observation that the induced ordered phase occurs only above a certain x-ray dose rate rather than accumulated dose is consis-

tent with a reversible switching process (see the figure). The large equilibrium spacing observed by the authors seems to result from repulsive filaments in a confined geometry, reminiscent of a two-dimensional Wigner crystal [where minimization of potential energy at low concentration leads to a two-dimensional crystal (3)].

Radiation-induced structural changes are usually detrimental. The term “radiation damage” is widely used to describe the resulting structural degradation. What is unusual and interesting in Cui *et al.*'s study is that x-ray irradiation induces rather than destroys ordering. The system seems to be highly susceptible to hexagonal order due to the built-in electrostatic repulsive force; above a critical concentration, bundles form spontaneously without x-ray radiation. By increasing the charge of the filaments, irradiation tips the transition point to much lower concentrations, where spontaneous bundling does not occur.

Further studies are needed to clarify the detailed x-ray-induced ionization process responsible for the ordering observed by Cui *et al.* Nevertheless, the x-ray switch introduced by this study opens up entirely new

directions in nanoscale assembly. We expect that future work will extend the discovery to other systems, such as other peptide-based geometric shapes, including sheets and spheres. Other new directions may involve using grazing-incidence x-ray irradiation for the controlled growth of ultrathin ordered phases at interfaces.

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## ATMOSPHERIC SCIENCE

# A Test for Geoengineering?

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Scientific and political interest in the possibility of geoengineering the climate is rising (1). There are currently no means of implementing geoengineering, but if a viable technology is produced in the next decade, how could it be tested? We argue that geoengineering cannot be tested without full-scale implementation. The initial production of aerosol droplets can be tested on a small scale, but how they will grow in size (which determines the injection rate needed to produce a particular cooling) can only be tested by injection into an existing aerosol cloud, which cannot be confined to one location. Furthermore, weather and climate variability preclude observation of the climate response without a large, decade-long forcing. Such full-scale implementation could

disrupt food production on a large scale.

We use the term “geoengineering” to refer to solar radiation management (SRM), particularly the injection of aerosols into the stratosphere to emulate volcanic emissions. We consider the best case for conducting experiments in the atmosphere, putting aside some of the worst-case reservations that have been raised about the atmospheric risks of geoengineering (2, 3).

If ongoing climate modeling and limited experiments to test insertion methodology were to indicate that SRM would reverse many negative aspects of global warming, could these results be validated with in situ experiments to test the creation of a stratospheric aerosol cloud and the resulting climate response? Some authors have argued that the effects of polar testing could be confined to the Arctic (4). However, we have shown (5), on the basis of analogs from past volcanic eruptions and climate model experiments, that Arctic injection would cool the atmosphere down to latitude 30°N, weakening the summer monsoon over Africa and Asia

Stratospheric geoengineering cannot be tested in the atmosphere without full-scale implementation.

and reducing precipitation, just like tropical injections of stratospheric aerosols. Indeed, any high-latitude sulfate aerosol production would affect large parts of the planet.

Even if insertion does indeed have to end up as planetwide, it might be thought that one could at least proceed at low rates of insertion and look for any untoward side effects before increasing the dose. But two major issues prevent useful testing of stratospheric aerosol injection with small amounts.

First, to produce an aerosol cloud of sufficient thickness that lasts long enough to detectably cool Earth's surface, regular injections would be needed into air that already contains an aerosol cloud. One can fly aircraft or balloons into the stratosphere and test nozzles and injection of material into the wake of the planes (see the figure) (6), and thereby measure the creation of aerosols in the first minutes or hours into a pristine stratosphere. However, current theory tells us that continued emission of sulfur gases or sulfate particles would cause existing particles to grow to larger sizes, larger than volcanic eruptions typ-

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ically produce. These larger particles would be less effective at cooling Earth, requiring even more injections (7). Such effects could not be tested except at full scale.

Second, the signal of small injections would be indistinguishable from the noise of weather and climate variations. The only way to separate the signal from noise is to get a large signal from a large forcing, maintained for a substantial period. Different model simulations [e.g., (5)] have shown that injection of 5 Tg ( $5 \times 10^{12}$  g) of  $\text{SO}_2$  into the tropical lower stratosphere every year—the equivalent of one 1991 Mount Pinatubo eruption every 4 years—could lower global average surface air temperature, but African and Asian summer precipitation would also be reduced, potentially affecting the water and food supplies of more than 2 billion people. If much less  $\text{SO}_2$  were injected, any potential effect on the monsoon would be indistinguishable from climate noise.

Volcanic eruptions serve as an excellent natural example of this. In 1991, the Mount Pinatubo volcano injected 20 Tg of  $\text{SO}_2$  into the stratosphere (8). The planet cooled by  $\sim 0.5^\circ\text{C}$  in 1992 and then warmed back up gradually as the volcanic cloud fell out of the atmosphere over the next year or so. There was a large reduction of the Asian monsoon in the summer of 1992 (9) and a measurable ozone depletion in the stratosphere (10). The eruptions of the Kasatochi volcano in 2008 (1.5 Tg of  $\text{SO}_2$ ) and the Sarychev volcano in 2009 (estimated 2 Tg of  $\text{SO}_2$ ) did not produce a climate response that could be measured against the noise of chaotic weather variability.

Climate model simulations suggest that the equivalent of one Pinatubo every 4 years would be required to counteract global warming for the next few decades. The cloud would have to be maintained in the stratosphere to allow the climate system to cool in response, unlike for the Pinatubo case, when the cloud

fell out of the atmosphere before the climate system could react fully. Such an experiment would essentially be implementation of geoengineering. No matter what the results, it would be difficult to stop such an experiment quickly. First, all model simulations conducted so far, starting with Wigley (11), show that upon cessation of geoengineering, the climate would warm much more rapidly than if no geoengineering had been conducted. This rapid warming would be much more disruptive than the gradual change we are experiencing now. Second, the geoengineering infrastructure, including different industrial interests involving many jobs, would lobby to keep the program going.

Furthermore, no stratospheric aerosol observing system exists to monitor the effects of any in situ testing. After the 1991 Pinatubo eruption, data from the Stratospheric Aerosol and Gas Experiment II (SAGE II) instrument on the Earth Radiation Budget Satellite showed how the aerosols spread. A limb-scanning design such as that of SAGE II is optimal for measuring the vertical distribution of aerosols. SAGE III flew from 2002 to 2006, but there are no plans for a follow-on mission. A spare SAGE III sits on a shelf at a NASA lab and could be used now. The only limb-scanner currently in orbit, the Optical Spectrograph and Infrared Imaging System (OSIRIS) on the Odin satellite, is not used to regularly monitor stratospheric aerosols. These current and past successes could serve as a model for a robust stratospheric observing system, which could also be used to measure the effects of episodic volcanic eruptions.

Finally, local impacts are particularly difficult to predict. Modeling to date has raised concerns that large-scale sulfur insertion might produce untoward local climate responses affecting both temperature and moisture. Global climate models sim-

**Potential techniques for producing aerosols in the stratosphere.** The effects of creating aerosols in a pristine stratosphere can be tested, but longer-term effects as the particles grow to larger sizes are difficult to predict and attribute.

ulate monsoon circulation at a fairly large scale. The more local the interest, the longer an experiment would have to run to rule out adverse side effects. In a 10-year experiment to test for a climate signal over noise, the chance of a local adverse response could not be ruled out prior to the experiment. As such, a prudently designed experiment would have to make provision for such outcomes. Although even a major disruption of agricultural output would be difficult to attribute to geoengineering, were such outcomes to occur, necessitating an end to the experiment, the sulfate aerosol density would need to be decreased slowly to avoid ecological shocks. All these issues will need to be considered in policy and governance deliberations (1).

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## ANTHROPOLOGY

# Apes Among the Tangled Branches of Human Origins

Terry Harrison

The detailed description of *Ardipithecus ramidus* (1) more than lived up to the buzz of anticipation that preceded it in the paleoanthropological community. *A. ramidus* is a purported hominin (the group comprising humans and their extinct relatives after they diverged from our closest living relatives, the chimpanzees) from the Middle Awash region of Ethiopia. The focus of attention has been on how *A. ramidus* may relate to later fossil hominins and to living apes and humans (see the first figure), but to appreciate the place of *A. ramidus* in human origins, we must also view it from the perspective of the hominoids (apes) that lived in the Miocene, 23 to 5 million years ago (see the second figure).

*A. ramidus* is known from more than 100 specimens, including a remarkably preserved partial skeleton, that date back to 4.4 million years ago (2). Several other hominin contenders are known from the late Miocene (7 to 5 million years ago), including *Ardipithecus kadabba*, *Orrorin tugenensis*, and *Sahelanthropus tchadensis*, but our knowledge of

their anatomy is much less complete. Not all paleoanthropologists (including this author) accept that *A. ramidus* is a hominin or agree with the evolutionary and paleobiological interpretations that have been proposed (2), but there is no doubt about its critical importance for understanding human origins (3, 4). The unveiling of *A. ramidus* has required a major rethinking of what the last common ancestor of humans and chimpanzees looked like and which initial evolutionary steps may have characterized the earliest hominins. *A. ramidus* also helps to close the gap between the last common ancestor of humans and chimpanzees (estimated at 7 to 5 million years ago) and the earliest undoubted hominin, *Australopithecus anamensis* (4.2 million years ago) (5).

During the early Miocene (23 to 16 million years ago), the precursors of hominoids—the proconsuloids—were a remarkably diverse group of catarrhine primates (the group comprising Old World monkeys and apes, see the first figure) restricted to the tropical forests and woodlands of Africa and the Arabian Peninsula (6). Between 17 and 14 million years ago, environments in Africa became drier and increasingly more seasonal. Proconsuloid diversity declined, and cercopith-

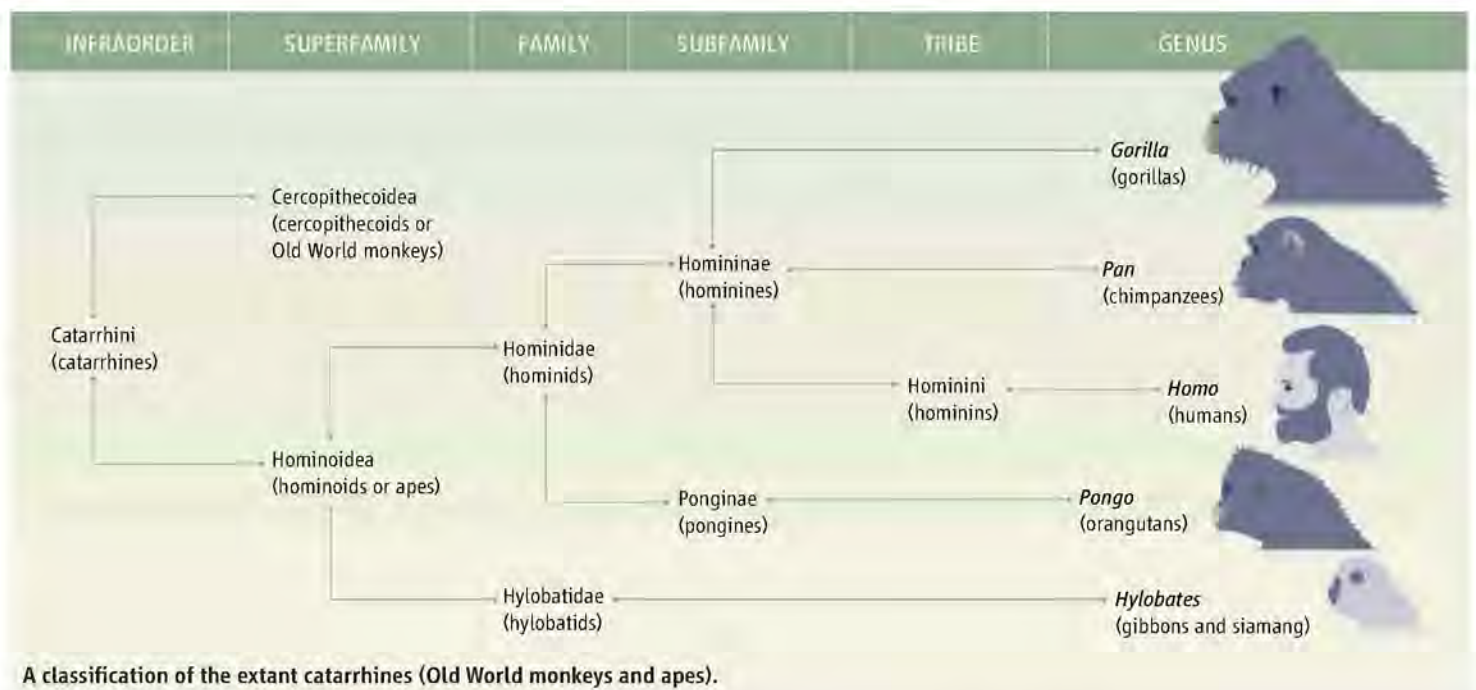
The evolution of apes between 23 and 5 million years ago set the scene for the emergence of the first hominins in Africa.

ecoids (Old World monkeys) and early hominoids, such as *Kenyapithecus*, *Equatorius*, and *Nacholapithecus*, became the dominant taxa (see the second figure). These hominoids and other catarrhines responded to increased seasonality by developing dietary adaptations for eating leaves or for processing hard food items, and by developing a range of specialized locomotor behaviors (6–8).

About 16 to 15 million years ago, apes expanded their geographic range out of Africa to colonize much of Eurasia. This influx of hominoids into Eurasia coincided with the middle Miocene climatic optimum, a phase of global warming that allowed tropical and subtropical mammals to extend their ranges northward. The earliest Eurasian apes, *Griphopithecus* and *Kenyapithecus*, are known from sites in Turkey and central Europe. Like their African contemporaries, they had thick-enameled molars and robust jaws, adaptations for exploiting a broad spectrum of seasonally available foods.

Between 13 and 9 million years ago, hominoid diversity in western and central Europe increased to include *Pierolapithecus*, *Anoiapithecus*, and at least four species of *Dryopithecus* (9, 10). *Pierolapithecus* and *Anoiapithecus* from Spain are probably stem hominids

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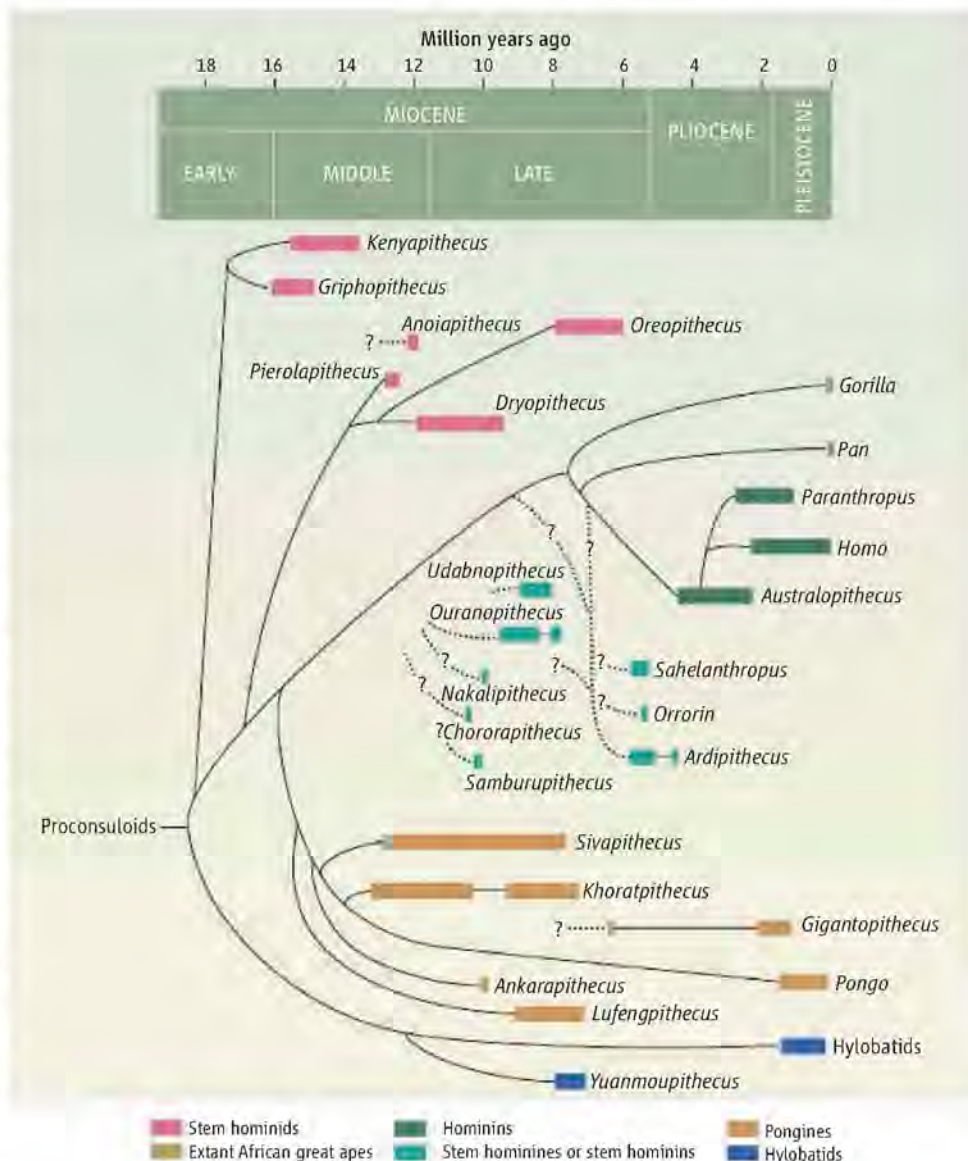
(great apes and humans, see the first figure). *Dryopithecus* has been inferred to be a stem hominid, an early member of the orangutan lineage, or a stem hominine (African great apes and humans, see the first figure), but the first of these options is the most plausible.

A diversity of hominoids also occurred in Asia during the middle and late Miocene, extending from Indo-Pakistan to Thailand. Of these, *Ankarapithecus*, *Sivapithecus*, *Lufengpithecus*, *Khoratpithecus*, and *Gigantopithecus* are all likely to be closely related to the extant orangutan (11).

Gradual cooling during the middle Miocene led to greater seasonality in western and central Europe and a shift from subtropical evergreen forests to predominantly deciduous broadleaved woodlands. This shift was accompanied by a dramatic turnover of the mammalian fauna at 9.6 million years ago, termed the Mid-Vallesian Crisis, when most hominoids became extinct (12). The highly specialized stem hominid *Oreopithecus* survived on European island refugia until 6 to 7 million years ago. In southeast Europe and southwest Asia, hominoids specialized for dry open woodlands, including *Ouranopithecus* and *Udabnopithecus*, survived well into the late Miocene (10 to 7 million years ago). *Ouranopithecus* probably offers the best evidence of an early hominine in Eurasia, which implies that African great apes extended their range from Africa into southeast Europe and southwest Asia about 10 million years ago.

About 7 to 8 million years ago, uplift of the Tibetan Plateau and increased intensity of the Asian monsoon, together with the global expansion of  $C_4$  grasses, led to a further decline in the diversity of Eurasian hominoids. By 5 million years ago, hominoids had become extinct throughout Eurasia, except for those surviving in the present-day range of Asian hominoids (orangutans and hylobatids, see the first figure), extending from southern China to Southeast Asia.

In Africa, the fossil record for hominoids between 13 and 7 million years ago is relatively sparse. This has led some authors to postulate that the hominines initially diverged in Eurasia before migrating back into Africa (13, 14). However, recent discoveries and a growing appreciation of later Miocene hominoid diversity in Africa make this an untenable scenario. The recently described 10-million-year-old *Nakalipithecus* from Kenya is closely related to *Ouranopithecus* but is older and has more primitive teeth, implying that these taxa shared a last common ancestor in Africa (15). It has been suggested that *Samburupithecus* (9.5 million years old) from Kenya and *Chororapithecus* (10 to 10.5 million years old)



**Hominoid relationships.** Schematic representation of the inferred evolutionary relationships between Miocene apes, early hominins, and extant hominoids. Solid gray bars represent the known time range of each genus, thin dark lines are inferred relationships between the genera, and thin dashed lines with “?” denote uncertain relationships.

from Ethiopia are related to gorillas, but the evidence is slim, and they are probably stem hominines (16). Further recent fossil finds confirm that in the middle and late Miocene, Africa was populated by a multitude of hominoids, but the current material is too scanty to designate additional species (15, 17, 18).

As paleontological exploration intensifies across Africa, our knowledge of hominoids in this critical time period will steadily grow. Rather than just a few relictual evolutionary strands surviving to the end of the Miocene and giving rise to modern hominine lineages, as was previously thought, ape diversity in Africa during the late Miocene looks very bushlike. The relationships between *Ardipithecus* and earlier hominids will remain enigmatic until the quality of the fossil evidence

from the late Miocene of Africa improves, but this will eventually prove critical in resolving its affinities to later hominins. The important questions then become: Where did *Ardipithecus* and the other early hominin contenders come from? Are they truly members of the hominine lineage, or simply apes among the tangled branches that constitute the basal hominine bush?

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## ECONOMICS

# Measuring Subjective Well-Being

Richard Layard

How should human happiness and life-satisfaction be assessed?

What is progress, and how should we measure the well-being of a population? The Organization for Economic Cooperation and Development has held two major conferences on the subject, and last year, President Sarkozy of France established a distinguished commission to report on the same questions (1). This major debate reflects the fact that higher national income has not brought the better quality of life that many expected, and surveys in the United States show no increase in happiness over the past 60 years. These surveys rely on questions about subjective well-being, and it is reasonable to ask how reliable survey answers are as measures of the quality of life as people experience it. On page 576 of this issue (2), Oswald and Wu carry out an interesting test of this. First they measure subjective well-being in each U.S. state, and then compare it with the average objectively measured wage in the same U.S. state (both variables being controlled for personal factors). The negative correlation of the two variables is remarkably high—as it should be if higher wages are compensating for a lower experienced quality of life (and vice versa). The study will likely stimulate some lively debate across many disciplines, including scientists, economists, sociologists, psychologists, and policy-makers.

But should we really adopt subjective well-being as our measure of the quality of life? Philosophically, many would say “yes,” as they have ever since the 18th-century Enlightenment. But, practically, can subjective well-being really be measured well enough to be used in policy analysis? Is what

people say about their subjective state well enough correlated with the inner reality?

The science is, of course, very young, but it is well enough developed for us to say “yes.” In the typical question, an individual is asked, “Taking all things together, how happy are you?” The possible answers range from 0 (extremely unhappy) to 10 (extremely happy). To evaluate the information con-



tent in the answers to such questions, we can examine whether these answers are well enough correlated with other relevant factors. They are in fact well-correlated with at least five relevant sets of variables: the reports of friends; the plausible causes of well-being; some plausible effects of well-being; physical functioning, such as levels of cortisol; and measures of brain activity.

When a subject's friends are asked about the subject's happiness, the answers correlate well with the subject's own report. (Were it not so, human society would find it hard to function.)

Moreover, questions on happiness and life satisfaction have now been asked in hundreds of routine population surveys, and in multiple regressions within countries, the following causal factors are always important: physical health, family status, employment, income, and age. This is true both in cross-section studies and in panel studies that include an individual fixed effect. Moreover, the sizes of the effects are remarkably similar in widely different studies done within different countries (3).

Similarly, responses on life satisfaction can be used to explain behavior such as quitting one's job and exiting from marriage. They can also, as Oswald and Wu show, be used to measure quality-of-life differences across the United States in a way that is consistent with the pattern of wage differences.

Answers about happiness are also well correlated with measurements of bodily function, such as amounts of salivary cortisol, fibrinogen stress responses, blood pressure, heart rate, and (in some cases) immune system responses to a flu vaccination. These correlations hold across individuals, as in the famous cross-sectional study of British Whitehall civil servants (4), and also in some cases within the same individual over time.

Finally, there are reported correlations with brain activity across individuals, and within individuals over time. The best known of these is the correlation of positive affect with activity in the left dorsolateral prefrontal cortex (PFC) and negative affect with activity in the right dorsolateral PFC (5). This area of work is in its infancy but, if successful, it will reinforce the view that subjective experience is an objective reality. Because this is so often questioned, it is worth repeating the findings of Coghill (6), who applied the same very hot pad to

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the legs of many subjects; in that case, the reported pain varied widely and was well correlated with activity in the cortex.

There are, of course, many different ways to measure happiness and life satisfaction. Such measures can be based on a single question or (to reduce measurement error) on many questions, which can be combined into a single index using weights that reflect their average impact on answers to the single question. For most purposes, we would like the measurements to cover a substantial period of time, but this can also be achieved by repeated questioning. And as the Enlightenment tradition suggests, the questions should mainly aim at capturing pure affect (feeling), without too much involvement of judgment (7). Fifty years ago, there was considerable debate on how to measure depression, but by now this has become much less controversial. In all likelihood, the measurement of happiness will become similarly less controversial.

Now is the time for every government to collect data on a uniform basis on the happiness of its population. That was the most important of the recommendations of Presi-

dent Sarkozy's commission (although it also recommended better measurement of GDP). Once there is good information on levels of happiness, three things will be possible: the monitoring of trends, the identification of problem groups in the population, and the analysis of why some people are happy and others are not. To fully understand intercountry differences in happiness, we shall probably have to rely on biomarkers in addition to answers to verbal questions asked in different languages.

As for policy analysis, this has to be based on solid science, using either naturalistic data or controlled experiments. But the metric has to change. Today's cost-benefit analysis measures benefit in terms of the money that the beneficiary has shown he or she would be willing to pay for a change of state. But for many key areas of public policy, such measurements make no sense because little individual choice is involved—think, for example, of physical health, mental health, responsible parents, family stability, (un)employment, and community life. In these areas, we can get much better measures of the benefits of a policy change through direct measures of subjective well-being. It is therefore time

to begin developing an alternative system of cost-benefit analysis in which the units are units of subjective well-being.

But sound measurement will only become possible if social science (including psychology) takes as a prime objective the quantitative study of the determinants of well-being. Every survey of individuals should automatically measure their well-being, so that in time we can really say what matters to people and by how much. When we do, it will produce very different priorities for our society.

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## MATERIALS SCIENCE

# Turning Away from High Symmetry

John C. Crocker

A common sight in cold climates is the growth of crystalline ice “feathers” or “ferns” on a cold window pane. These structures are initiated by heterogeneous nucleation—the ice crystals form on the surface of a different substance, in this case, specks of dust. It is much harder to start crystal formation in pure fluids because the seed crystal must form spontaneously in solution (hence pure water can supercool and remain as a liquid for long times below its freezing point). Homogeneous nucleation is largely a mystery; observing small clusters of molecules directly is difficult, and their formation is a rare event. On page 560 of this issue, Meng *et al.* (1) report an exhaustive experimental study of the equilibrium cluster configurations in a model system consisting of microscopic plastic spheres that were designed to have a short-ranged, reversible attraction that drives them together (2). This

work underscores the subtle geometrical difficulties associated with crystal nucleation, and helps us to understand the “rules” by which nature self-assembles small structures and ultimately crystals.

In earlier work assembling clusters, Manoharan and co-workers (3) found that highly symmetric polyhedral clusters were the most stable in terms of bond energies. However, in their current work on clusters in thermodynamic equilibrium, the most common structures are typically the least symmetric. The essential difference lies in entropic effects—in thermal equilibrium, the most common clusters are not those with the lowest internal binding energy (4), but rather those with the lowest free energy, which is favored by higher entropy.

Entropy has been equated in popular culture with unavoidable disorder and decay, but its true nature can be appreciated in the context of self-assembly. In equilibrium, every possible way to build a cluster (having the same binding energy) will occur with the same probability—there are simply fewer

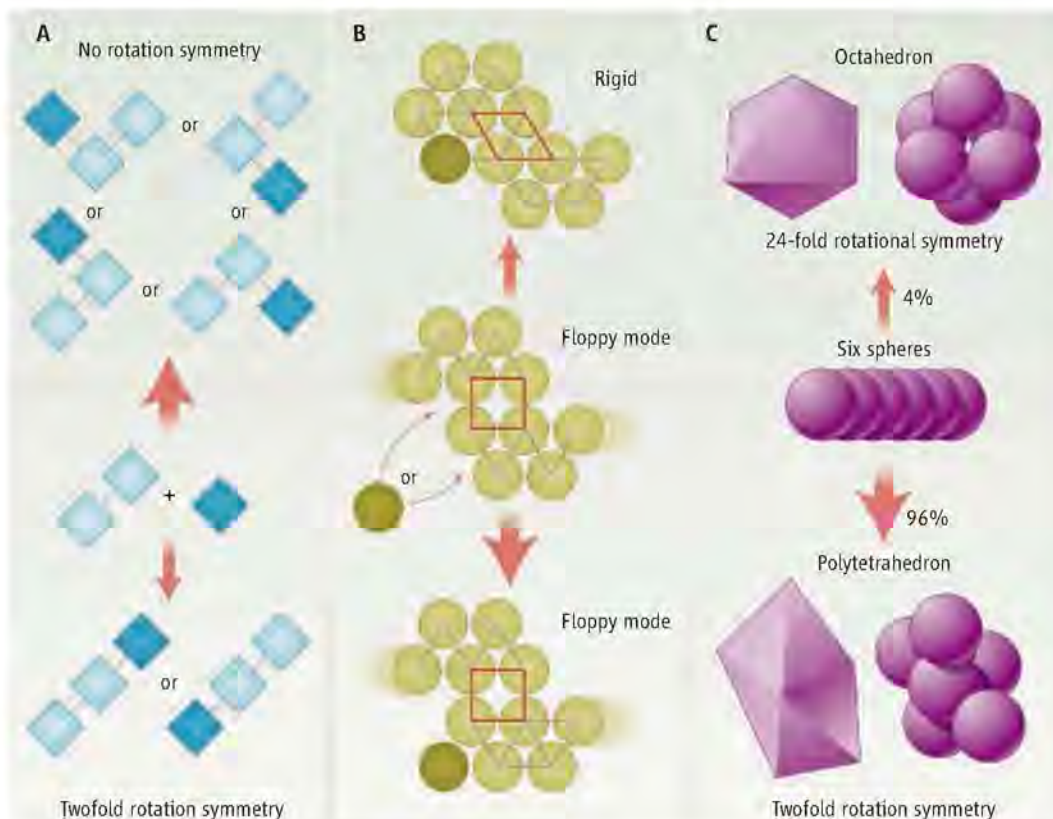
Light microscopy studies of cluster formation by colloidal particles show that less-symmetrical structures are favored under equilibrium conditions.

ways of building symmetric clusters (see the figure, panel A). Specifically, Meng *et al.* hypothesized that the symmetric polyhedra would encounter two entropy penalties. Their rotational symmetry leads them to have very low rotational entropy, and their high degree of internal coordination makes them very rigid, which causes them to have very low vibrational entropy (see the figure, panel B).

To experimentally test their hypothesis, Meng *et al.* first prepared a suspension of microspheres that are large enough to image with a light microscope, but small enough to undergo random thermal fluctuations and obey equilibrium statistical mechanics. This sample was then loaded into an array of microfabricated chambers, each of which was small enough that it would contain only a small number of microspheres. An additional entropic effect—called a depletion attraction—was induced between the microspheres by the addition of much smaller, nanoscale particles (2). This force causes the microspheres to join up to form clusters, one per chamber. Each cluster was then scrutinized

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**Let me count the ways.** A particular cluster is more likely to occur at equilibrium, because of entropic effects, when there are more ways to assemble the structure and when the structure has lower symmetry. **(A)** To understand rotational entropy, consider the simplified problem of adding a third “square” to a dimer of two squares (middle). There are four sites for adding the square that create a bent conformation (top), but only two sites for creating the linear conformation (bottom). The bent site is favored by having twice as many possible configurations, which is formally equivalent to its having half as many rotational symmetries. **(B)** To understand vibrational entropy, consider a cluster of disks that can assemble into rigid triangles or floppy squares, which flex to form a rhombus (middle). If a disk is added to the center structure adjacent to the square (top) when the square is fully flexed, it can be locked into and form two rigid triangles. The disk can also be add(ed?) elsewhere and leave the floppy mode intact (bottom). The rigid structure with lower vibrational entropy is less likely to form, as the time window for its formation is limited, whereas the binding sites that leave the floppy structure intact are always available. In equilibrium, the top cluster is far less likely to form than the bottom one. **(C)** When assembling six spheres, Meng *et al.* found that the most symmetric cluster, the octahedron (top), only occurs 4% of the time compared to 96% for the polytetrahedron (bottom). Rotational entropy leads to a factor of 12 difference in probability; the remaining factor of  $\sim 2$  is from differences in vibrational entropy.

under a microscope, and followed as it randomly tumbled end over end and diffused around its chamber by Brownian motion.

The difficult part lies in the determination of three-dimensional structure. Meng *et al.* recorded a short video for each cluster with enough frames to show the tumbling cluster from different angles. Then, like bird watchers identifying species, the authors matched each cluster’s distinctive appearance in the video, by eye, to a field guide of computer-generated images of cluster structures in different orientations. To collect a statistically complete sample of all clusters of up to eight spheres, they manually identified more than 500 clusters in this way. A partial survey of the structures with up to 12 spheres was also completed.

The hypothesis of Meng *et al.* was validated by this analysis: The most symmetric

and rigid structures were dramatically under-represented in their sample, in quantitative agreement with their free-energy model. In one example, clusters of six spheres can form two different structures with the same number of sphere-sphere bonds and binding energy: a regular octahedron, and a “polytetrahedron” consisting of three tetrahedra joined by adjacent faces (see the figure, panel C). Remarkably, the latter low-symmetry structure was observed 96% of the time, compared to just 4% for the octahedron. According to the authors’ model, this difference is almost entirely accounted for by the octahedron’s high rotational symmetry.

Similarly, the most symmetric and lowest-energy case for 12 spheres, the icosahedron, was never observed. For clusters of more than 10 spheres, structures containing both square pyramids and tetrahedra were favored relative

to pure polytetrahedra through a combination of high-entropy floppy modes (as illustrated in the figure, panel B) associated with the square pyramids and the higher degree of bonding possible for such structures. These structures also resemble familiar crystal symmetries, such as hexagonal close-packed (hcp).

The sphere-sphere attractions in this system are quite short-ranged and stiff compared to the microsphere diameter—the so-called sticky-sphere limit. The authors note that for the effectively longer-ranged potentials of atomic systems, their free-energy model predicts that the dramatic entropic effects become somewhat more subdued and the preferred cluster structures become temperature dependent. Generalizing their model to non-spherical molecules will be a non-trivial extension. Moreover, recent experiments with similar microsphere model materials have shown very slow (5, 6) or nonexistent (7) crystal nucleation, which seems inconsistent with the hcp-like cluster structures observed here.

Unlike the conditions here, where clusters can equilibrate at leisure in complete isolation, the clusters in an unbounded fluid are continuously bombarded by and grow by absorbing smaller clusters and can form transient gel-like chains of clusters, all of which may frustrate the equilibration of internal modes. It may well be that to understand nucleation, we need to

understand its absence—the breakdown of equilibration that prevails during the glass or gelation transition. Still, the field guide of equilibrium clusters provided by Meng *et al.* will provide a crucial baseline against which nonequilibrium clusters may be compared.

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## RETROSPECTIVE

## Edwin G. Krebs (1918–2009)

William A. Catterall and John D. Scott

Edwin G. Krebs, a giant of biomedical science in the 20th century, died on 21 December from congestive heart failure in Seattle at the age of 91. His discovery (with Edmond H. Fischer) of protein phosphorylation as a regulatory mechanism touched all aspects of biomedical science and profoundly influenced therapeutic approaches now used in clinical care. Ed's life epitomizes commitment to family, excellence in research, and academic service.

Edwin G. Krebs was born in Lansing, Iowa, in 1918, the son of a Presbyterian minister and a schoolteacher. His father died suddenly when Ed was 15 and, at the height of the Depression, the family moved to Urbana, Illinois, where Ed earned a degree in chemistry from the University of Illinois in 1940. As an undergraduate, he became enamored with organic chemistry but eventually chose to study medicine at the Washington University School of Medicine in St. Louis, Missouri. Although the principal responsibility of a medical school during World War II was to train physicians for the armed forces, Ed also participated in medical research. After medical school and residency training at Barnes Hospital in St. Louis, he went on active duty as a medical officer in the Navy. Following his discharge in 1946, he returned to St. Louis and was accepted as a postdoctoral fellow in the laboratory of Nobel Laureates Carl and Gerty Cori in the Department of Biochemistry. After 2 years of postdoctoral research on the interaction of protamine (a small sperm protein) with rabbit muscle phosphorylase, Ed became so captivated with biochemistry that he never returned to clinical medicine.

During his naval service, Ed enjoyed a brief visit to Puget Sound, so he happily accepted a position as assistant professor of biochemistry in the fledgling University of Washington School of Medicine in 1948. Under the visionary leadership of Hans Neurath, the

Department of Biochemistry expanded in protein chemistry and enzymology, including recruitment in 1953 of Edmond Fischer, a talented and charismatic Swiss biochemist studying potato phosphorylase. Thus, a lifelong friendship and a formidable research partnership were forged.

Together Ed (Krebs) and Eddy (Fischer) determined the mechanism by which adenosine 5'-monophosphate (AMP) served as an activator of phosphorylase b in skeletal muscle. They found that adenosine 5'-triphosphate (ATP) was required for phosphorylase activation, and in an unusual experiment discovered that calcium, leaching from filter paper used to clarify the muscle extract, was an important cofactor. They demonstrated that phosphate was incorporated into a specific serine residue of phosphorylase b, thereby yielding an activated form called

phosphorylase a. Their landmark paper was published in the *Journal of Biological Chemistry* in 1955. Subsequently, Krebs, Fischer, and colleagues confirmed that this phosphorylation is mediated by another enzyme (phosphorylase b kinase), which itself is controlled by yet another enzyme, a cyclic AMP (cAMP)-responsive kinase. This finding led directly to the concept of kinase cascades. In 1968, Ed purified the cAMP-dependent protein kinase (protein kinase A). The recognition that protein kinase A is the primary cellular effector for cAMP signaling placed protein phosphorylation in a central position in hormone action and broadened the importance of this protein modification in cell biology, physiology, and pharmacology.

At the same time as his discovery of protein kinase A, Ed's interests in teaching and academic administration led him to seek new opportunities and challenges as founding chair of the Department of Biological Chemistry at the University of California, Davis. He also embarked on a long association with the American Society for Biochemistry and Molecular Biology as associate editor of the *Journal of Biological Chemistry* for 20 years and as president of the American Society

Defining how protein phosphorylation is regulated led to an explosion of knowledge about most cellular processes.

for Biochemistry and Molecular Biology in 1985. In 1977, Ed returned to the University of Washington as investigator of the Howard Hughes Medical Institute and chair of the Department of Pharmacology, where he inaugurated a major new research direction in molecular pharmacology. What he liked most about both of his chair positions, he said, was the responsibility of selecting outstanding faculty members for the departments.

After achieving his goals as department chair in 1983, Ed refocused his efforts on research, training junior scientists, and solving new problems in cell signaling. His laboratory studied signaling events that involved the phosphorylation of proteins on tyrosine residues. His lab was also instrumental in the discovery of the mitogen-activated protein kinase pathway, a sequence of protein kinases that respond to extracellular stimuli and regulate a wide range of cellular processes including gene expression and cell growth, differentiation, and survival.

Krebs received many major scientific awards for his insights into the principles governing cellular regulation in health and disease, including election to the National Academy of Sciences (1973), the Passano Foundation Award (1988), the Horwitz Prize (1989), the Lasker Medical Research Award (1989), the 3M Life Sciences Award (1989), and the Welch Award in Chemistry (1991). At age 74, he and Edmond Fischer were honored with the 1992 Nobel Prize in Physiology or Medicine for their discovery of protein phosphorylation. In 1997, Ed finally closed his lab but was frequently spotted wandering the halls of the University of Washington Medical Center on his way to hear the latest research seminars. Ed is survived by his wife of 64 years Virginia (Deedy) Krebs, three children, and several grandchildren.

Edwin G. Krebs will be remembered for his keen intellect, astonishing research productivity, and iconic status within the research community. Those who were privileged to work closely with him will remember him fondly as a kind and gentle mentor who passed on extraordinary insights in a quiet and dignified manner. The legacy of this self-proclaimed reluctant biochemist should be a wonderful inspiration to the next generation of our profession.





# Making Genetics Easy to Understand

Louisa A. Stark\* and Kevin Pompei

The Human Genome Project and the subsequent explosion of genomic information are transforming our knowledge of how organisms function and how genes and the environment interact. These insights have led to advances in personalized medicine, stem cell treatments, and genetic testing. Students, teachers, and the public must be prepared to make informed decisions about participation in genomics research, genome-related health care, use of genetically modified agricultural products, and public funding for stem cell research. Education has been identified as a crosscutting element that is critical to achieving the potential of genomics research (1).

To address this need for genomic literacy, we have developed two related Web sites. Learn.Genetics (see figure, right, from <http://learn.genetics.utah.edu/>) provides educational materials that currently cover 15 topic areas ranging from DNA to epigenetics. Classroom activities designed to support and extend these materials, as well as other resources for educators, are available on Teach.Genetics (<http://teach.genetics.utah.edu/>).

Our goal in developing these Web sites has been to make genetics and genomics easy for everyone to understand. The grants and contracts funding the sites have supported development of curriculum-supplement materials for grades 5 through 12 that address the National Science Education Standards (2) and gaps in standards-related, free, online, multimedia educational materials.

Web-site and in-person feedback indicate that the materials are used by a much broader audience. Students from middle school through

graduate school use the site to better understand content their instructors present, to assist in completing assignments, and to explore science independently. Higher-education faculty use the materials for courses ranging from introductory biology to professional preparation in education and nursing.

Animations presenting science concepts in an accessible and engaging way attract members of the general public, which leads

An integrated pair of Web sites for students and teachers supports genetics and genomics education worldwide.

to “viral” dissemination through link-sharing Web sites and blogs. Although this type of dissemination is unpredictable, both our “Mouse party” and “Cell size and scale” (see figure below) interactive animations have spread this way, engendering discussions about science in over 30 languages around the world. “The new science of addiction: Genetics and the brain” module has received the most unanticipated use; it has been incorporated into police officer training and addiction treatment in several countries.

We use a participatory design approach to developing our materials, involving teachers and scientists along with the science educators, instructional designers, science writers, teacher professional developers, scientists, multimedia designers, Web developers, and evaluators that comprise our team. Our method emerged from extensive work with teachers in professional development programs and capitalized on teachers’ real-world expertise in successful teaching approaches, knowledge of engaging topics and materials, knowledge of the gaps in available online materials, and familiarity with the state science education standards guiding curricula. It also draws on scientists’ depth of expertise in their fields. Involving the center’s entire team builds understanding of the content and learning objectives and enables each to contribute his or her perspective and expertise to the process—from writing, visualization, production, classroom evaluation, and teacher professional development.

Our development process for a module begins with a summer workshop, advertised to teachers through our e-mail list. An online application enables us to select an outstanding group of 12 to 18 grade-appropriate teachers who represent a diversity of



**Learn.Genetics.** The site provides educational materials on 15 topic areas, ranging from DNA to epigenetics.



**Cell size and scale.** This interactive animation allows users to zoom from a coffee bean down to a carbon atom, comparing the relative sizes of representative cells, microorganisms, organelles and molecules along the way.

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teaching experience, student populations, and locales; about 5 to 10% of applicants are accepted. Participants receive travel expenses and a stipend.

A typical 4½-day summer workshop begins with talks by scientists and discussions of scientific articles, from which participants distill important concepts for their students. The teachers and our staff work together to define the “big ideas” that emerge from these concepts, around which the module will be organized. Small groups of teachers then develop each big idea, drafting online and classroom learning experiences designed to assist students in learning. The workshops offer a rare opportunity for teachers to develop creative ideas for curriculum materials that will be used worldwide, to interact with scientists, to update their content knowledge, and to work with other teachers from across the country. A glimpse into one summer workshop can be seen at <http://learn.genetics.utah.edu/content/epigenetics/credits/>. In it, teachers describe ideas that became the *Insights From Identical Twins* movie and “Gene control” interactive animation on Learn.Genetics and the “DNA and histone model” activity on Teach.Genetics.

After the summer workshop, our team works with the materials the teachers drafted. Ideas may be combined, modified, expanded or contracted, as we plan a module that addresses the big ideas while fitting the anticipated cost within the available budget.

Our materials evolve each year as we learn from past modules and feedback obtained via classroom testing, teacher workshops, and Web-site feedback. At present, key module concepts are addressed in animated and interactive activities, because these appeal to the broadest range of learners. Narration allows users to concentrate on the animation and leads to deeper learning (3); text is available for those who are hearing-impaired or in computer labs lacking headphones. “Learn More” pages provide additional information for those interested in exploring beyond the basics. All pages are designed following standard Web usability guidelines (4), including meaningful visuals and text that is clear, concise, and easily scanned.

Good instruction addresses multiple learning styles, such as visual, auditory, and kinesthetic (5, 6). Therefore, our modules include non-computer-based classroom materials designed to support, extend, and assess online learning. For example, the somatic cell nuclear transfer (SCNT) technique utilized in the “Click and clone” interactive animation is mirrored in the



**Kevin Pompei** is the GSLC associate director and leads the center’s educational material development team. He has served as CEO of two software companies and has extensive experience in software and Web design, Web architecture, and database development, as well as managing teams of diverse technology professionals. He is working on a graduate degree in Educational Psychology with a focus on Instructional Design and Educational Technology.



**Louisa A. Stark, Ph.D.**, is director of the Genetic Science Learning Center (GSLC) at the University of Utah. During graduate school, Stark had the opportunity to take hands-on science into Denver inner-city schools as a member of the “Science Squad,” sparking a passion for working with kindergarten through grade-12 students and teachers. After completing her doctorate in evolutionary genetics, she began a career with science education partnership programs, joining the GSLC in 1999. Her recent awards include the 2008 Friend of Science Education Award, the 2008 Award for Excellence in Human Genetics Education, and a 2009 Distinguished Alumni Award.

paper-based “Let’s clone a mouse, mouse, mouse” activity. We suggest using the latter as an assessment for the online activity, asking students to use the mouse and petri dish paper cut-outs to create a poster describing the SCNT technique, without giving them the instructions. Additionally, worksheets are provided for many other animations to guide students’ learning.

An entire module comprises 2 to 10 hours of instruction. However, in designing our materials, we recognize that many teachers do not use a curriculum supplement module in its entirety (7). They select materials that address the science standards they are required to teach, are appropriate for their students’ level, and fit their instructional designs (7). For example, in the “Amazing cells” module, a seventh-grade teacher might only use “Inside a cell” and “Cell size and scale.” A high-school biology teacher might use these activities plus “Build-a-membrane,” “Coffee to carbon,” and “The fight-or-flight response.” To address multiple grade levels and teachers’ differing use of the curricula, each animation and classroom activity focuses on a single main learning objective, making it easier for teachers to incorporate the materials into their lessons.

As our team produces a module, they consult the scientists who participated in the summer workshop and others for additional information and to verify scientific accuracy. Currently, module production also includes development of valid and reliable assessment instruments, used in classroom field tests and later added to the resource materials on Teach.Genetics. A medium-sized module, such as “Epigenetics,” takes 3 to 4 months to produce.

Once a module is produced, it is tested in the classroom with students and teachers who were not involved in the development process. Students complete knowledge assessments before, after, and 2 weeks after studying a module. Teachers receive a stipend for their participation and feedback.

We have begun expanding beyond genetics with our “Amazing cells” and “Great Salt Lake ecology” modules. We hope to develop additional materials in the areas of life science and health and other scientific fields.

The Web has become the primary information source for individuals with access to the Internet. Online educational materials thus have the ability to affect science literacy, preparing individuals to participate in the workforce, as well as to become informed health-care consumers and citizens of the 21st century.

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## ASSOCIATION AFFAIRS

## Alice Huang: Passion, Freedom Are Crucial to Global S&T Progress

The old year was ending and a new one beginning, and Alice Huang was scanning the popular news media lists of the most influential people of 2009 and the decade past, hoping to see a scientist or an engineer. This year, she didn't find one.

Though there are many worthy candidates, the lack of public recognition defines a crucial challenge for American science, the incoming AAAS president said in a recent interview. Scientists and science teachers must do more to convey to the public—and to science students—the idealism, creativity, and passion that drive many breakthroughs.

Huang, a distinguished virologist now at the California Institute of Technology, described how the thrill of her own first discoveries inspired a career of research. But too often these days, she said, even excellent students lack that passion.

"We don't focus on that when we first talk to people about science," Huang said. "When young people come to me and ask for advice, I find that they're very focused on 'how do I get ahead, and how do I get the position I want?' Somehow they've forgotten...the joy of living a passionate life. Every scientist I've met who is successful is indeed passionate about what they do."

In Huang's view, talking about passion and idealism is crucial for addressing challenges in health, energy, the environment, and other fields. It can help build public understanding of science and support for investment in research and development. It can help recruit more students into science and engineering, especially from the underutilized pool of women and minorities. Overseas, it can help drive home the point that a culture of freedom is critical to scientific advancement.

Huang knows her subject well—since earning her Ph.D. at Johns Hopkins in 1966, she has come to occupy an influential position at the juncture of research, education, and diplomacy.

As a graduate student, Huang discovered and characterized defective interfer-



**Incoming AAAS president.** Alice S. Huang, a senior faculty associate in biology at Caltech, will become the president of AAAS on 22 February, when the association's annual meeting closes in San Diego.

ing viruses—viruses that have the potential to help control viral diseases in plants, animals, and humans. Her work raised the possibility that defective interfering viral particles could be used for disease prevention. Her postdoctoral work with David Baltimore at the Salk Institute and MIT on vesicular stomatitis virus led the way to Baltimore's Nobel Prize-winning discovery of reverse transcriptase.

Huang spent 20 years on the faculty at Harvard Medical School; from 1979 to 1990, she also directed the Laboratories of Infectious Diseases at Children's Hospital in Boston. She was appointed dean of science at New York University in 1991, and in 1997 moved west to serve as senior councilor for external relations at Caltech. Today, she's a senior faculty associate in biology there.

Huang is a past president of the American Society for Microbiology, and served from 2004 to 2009 on the California Council on

Science and Technology. She has consulted on science policy for government agencies in China, Taiwan, and Singapore; at the Singapore Botanic Gardens, there's a species of orchid named for her.

Born in the city of Nanchang in southeastern China, Huang came to the United States with her family as a young girl. Her father had been bishop of the Anglican Episcopal ministry in Southwest China, but if not for his calling to the clergy, he told her, he might have been a doctor. Her mother was a nurse.

From age 7, Huang set out to become a physician. Attending parochial girls' schools, "I had some wonderful teachers," she recalled. "They understood why I was curious about things and gave me the tools to realize the answers to my curiosity."

It was at Johns Hopkins that she was first exposed to scientific research, and that diverted her from medicine. But she was involved in medical research for much of her career, working on viruses, cancer, HIV, and other diseases.

She served on the AAAS Board of Directors from 1997 to 2001 and was elected a AAAS Fellow in 2000. She will succeed Nobel laureate Peter Agre as president when the AAAS Annual Meeting closes on 22 February; Agre will begin a 1-year term as chairman of the AAAS Board.

Huang sees AAAS and *Science* as strongly positioned to support the scientific enterprise on issues ranging from political manipulation of data to reducing laboratories' paperwork requirements. She cited two priority areas for continuing AAAS efforts: supporting women and minorities, and international science engagement.

International science collaboration can propagate a scientific culture based on free inquiry and meritocracy, Huang said. "The unfettering of curiosity and freedom to ask questions in pursuit of scientific discoveries can become a subtle force for a freer society and less authoritarian governing system."

In the United States, science must help women and minorities break through the glass ceiling to leadership positions. "They're generally hired into the profession and not promoted in proportion to their numbers," she said. "It's a tragic loss of individuals who have the talent and capability and who really have the ability to contribute to our society."



## Science Books & Film Gets Digital Makeover

After 44 years in print, AAAS's *Science Books & Film* has gone online with a host of new features, ensuring the venerable journal's position as one of the world's leading authorities on science media resources.

Podcast interviews with authors, a weekly editor's blog, and a searchable database of thousands of reviews are among the valuable new tools for librarians, parents, and readers. Although best known for its reviews of children's books, *SB&F* also covers science television and film, software, and Web sites.

"*SB&F* has been a one-of-a-kind journal for decades," said Heather Malcomson, the journal's editor for the past 8 years. "But I think that the online presence, using a number of platforms, will really give us an opportunity to reach more people, and more young people. We know the enthusiasm for science reading is out there, and we want to connect those readers with the best the science world has to offer."

Each monthly online issue, available at [www.sbfonline.com](http://www.sbfonline.com), contains about 75 reviews written by scientists, educators, and media specialists. But the new blog for subscribers expands these offerings with a weekly look at new books and software, science on television, and ongoing *SB&F* projects. Malcomson hopes that the blog will also "become an interactive place for me to communicate with our readers."

Librarians, who rely on the reviews to guide critical purchasing decisions for their science sections, make up the bulk of more than 1500 journal subscribers, according to Malcomson. Patrons of many public libraries, as well as individual subscribers, have full access to all of *SB&F*'s features, including lists of the past year's Best Books for Children, Junior High, and High School Students, and Best Video and Software.

Subscribers can also plan their monthly viewing schedule with the help of *SB&F*'s "Science on TV" column, which offers short descriptions of select programs with a scientific bent.

Even without a subscription, visitors to *SB&F* online can listen to the journal's podcast series, "AAAS Book Talks." The series, supported by the William T. Golden Endowment Fund for Program Innovation, interviews award-winning science authors to learn more about their inspiration and future projects.

"The authors have really interesting stories to share about where the idea for the book came from or stories behind the book, like research expeditions," said Malcomson, who does many of the interviews along with *SB&F* Editor-in-Chief Maria Sosa. "All of the

authors have a desire to communicate science in a way that is exciting and encourages listeners to take an interest in science."

Along with auto manufacturer Subaru, *SB&F* sponsors an annual award honoring individual science books. The AAAS/Subaru *SB&F* Prizes for Excellence in Science Books celebrate outstanding science writing and illustration for children and young adults.

—Molly McElroy and Becky Ham

### AAAS

## Call for Nomination of 2010 Fellows

AAAS Fellows who are current members of the association are invited to nominate members for election as Fellows. A Fellow is defined as a member "whose efforts on behalf of the advancement of science or its applications are scientifically or socially

distinguished." A nomination must be sponsored by three AAAS Fellows, two of whom must have no affiliation with the nominee's institution.

Nominations undergo review by the steering groups of the association's sections (the chair, chair-elect, retiring chair, secretary, and four members-at-large of each section). Each steering group reviews only those nominations designated for its section. Names of Fellow nominees who are approved by the steering groups are presented to the AAAS Council for election.

Nominations with complete documentation must be received by 10 May 2010. Nominations received after that date will be held for the following year. The nomination form and a list of current AAAS Fellows can be found at [www.aaas.org/aboutaaas/fellows](http://www.aaas.org/aboutaaas/fellows). To request a hard copy of the nomination form, please contact the AAAS Executive Office, 1200 New York Avenue NW, Washington, DC, 20005, USA; at 202-326-6635; or at [gsciler@aaas.org](mailto:gsciler@aaas.org).

### ANNUAL MEETING

## Seeking Global Solutions, Scientists Meet in San Diego

The AAAS Annual Meeting has long been known as the premier multidisciplinary science gathering in the United States, but this year it will continue its evolution to a prime international affair: When the meeting convenes in San Diego from 18 to 22 February, scientists, journalists, and educators from more than 50 nations will be there.

In his invitation to the meeting, AAAS President and Nobel laureate Peter C. Agre urged his colleagues to embrace a global vision of science. The meeting "calls on every scientist and engineer to make their work both beneficial and understandable," said Agre. "It is a call to action that resonates around the world."

Convening under the banner "Bridging Science and Society," top researchers will discuss their findings in the context of global challenges in the environment, economy, health, and education. Attendees can explore cutting-edge research in the neurosciences, energy, astrobiology, public health, and environmental change, and learn how these advances directly affect courtroom trials, care for the elderly, sustainable cities, border security, and other public concerns. Experts will also discuss efforts to

open up laboratories and factories and bring research to the classroom in an unprecedented effort to show students the day-to-day world of discovery and application.

Agre will open the 176th Annual Meeting with a presidential address; other prominent speakers include U.S. presidential adviser Eric Lander, who will discuss science and technology in the administration of U.S. President Barack Obama; Nobel laureate Carol Greider, who will talk on the genetics of degenerative disease; U.S. Geological Survey Director Marcia McNutt, who will speak on ocean science; and International Linear Collider Director Barry C. Barish, whose address will focus on new frontiers in particle physics.

The staff of *Science*, *ScienceNOW*, and AAAS's *Science Update* radio program will provide coverage from San Diego in news reports, podcasts, and blogs. AAAS.org's Annual Meeting News Blog will provide extensive reporting on symposia and briefings, along with links to

U.S. and international news coverage. Find the full spectrum of Annual Meeting coverage at [www.aaas.org/go/news](http://www.aaas.org/go/news).

For registration and other information about the 2010 Annual Meeting, visit [www.aaas.org/meetings](http://www.aaas.org/meetings). Information from the meeting will also be posted at the 2010 AAAS Annual Meeting and *ScienceNOW* pages on Facebook, and via Twitter at #AAAS10.

—Becky Ham





# Coexistence of Quiescent and Active Adult Stem Cells in Mammals

Linheng Li<sup>1</sup> and Hans Clevers<sup>2</sup>

Adult stem cells are crucial for physiological tissue renewal and regeneration after injury. Prevailing models assume the existence of a single quiescent population of stem cells residing in a specialized niche of a given tissue. Emerging evidence indicates that both quiescent (out of cell cycle and in a lower metabolic state) and active (in cell cycle and not able to retain DNA labels) stem cell subpopulations may coexist in several tissues, in separate yet adjoining locations. Here, we summarize these findings and propose that quiescent and active stem cell populations have separate but cooperative functional roles.

Stem cells constitute a long-lived population of cells that possess the ability to self-renew (a process of duplication without losing developmental potential) and give rise to multiple cell types (differentiation) (1). In *Drosophila* and *Caenorhabditis elegans*, a single population of germ stem cells (GSCs) resides in a single niche (2, 3). The asymmetric architecture of the stem cell niche dictates that stem cells normally divide asymmetrically into a new stem cell (self-renewal) and a committed progenitor (differentiation). GSCs only divide symmetrically (self-renewal only) when expansion of the stem cell pool is required (3). The invertebrate GSCs normally undergo constant cycling (either asymmetric or symmetric division) (4, 5) and become quiescent when animals are challenged nutritionally (6). In contrast, mammalian adult stem cells are generally detected as in a predominantly quiescent state (7–9). How can long-term quiescent stem cells support rapidly regenerating tissues (e.g., producing billions of blood and intestinal cells daily) during normal physiology? The mechanism whereby quiescent stem cells give rise to transit amplifying (TA) cells, which in turn differentiate into mature cells, may not provide a satisfactory answer because TA cells are short-lived and cannot self-renew. Recently, populations of stem cells that are long-lived yet constantly cycling have been identified (10). Here, we integrate insights from bone marrow, intestinal epithelium, and hair follicle to formulate an alternative and complementary model in which subpopulations of quiescent and active adult stem cells coexist in the same tissue.

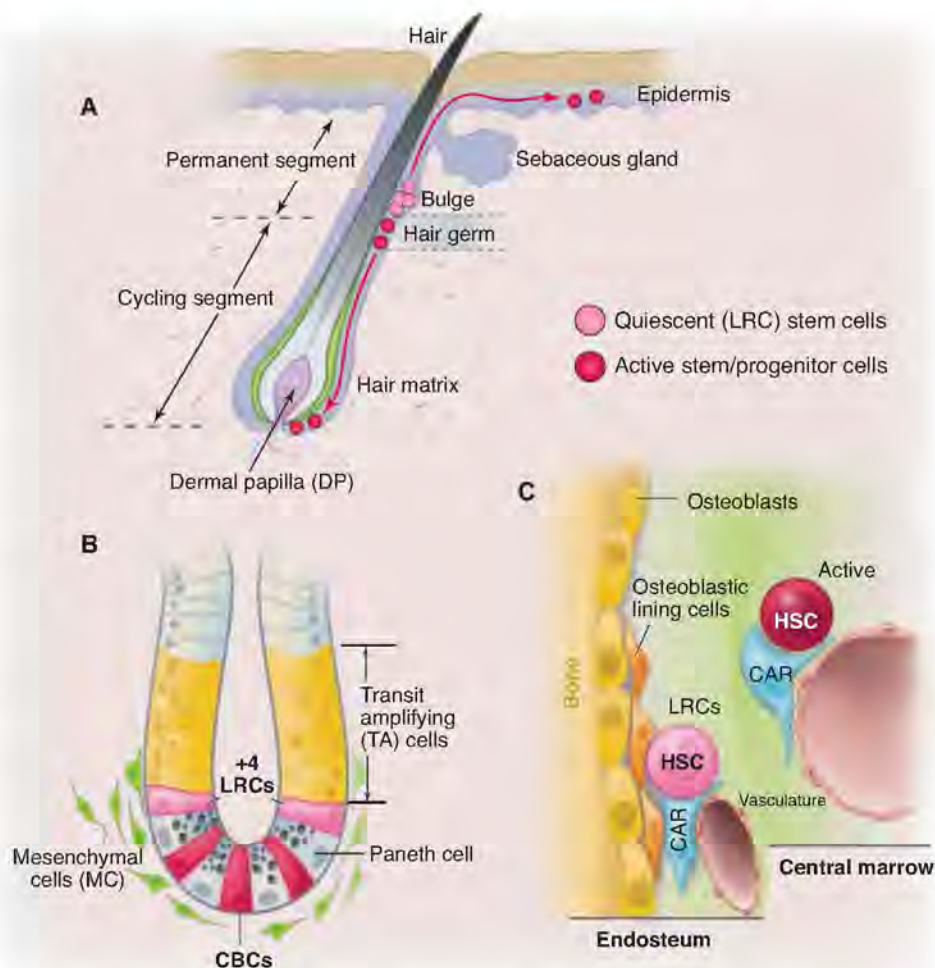
## Hair Follicle

The hair follicle provides an excellent system for studying stem cell biology. Each hair follicle is composed of a permanent portion, which in-

cludes the sebaceous gland and the underlying bulge region, and a temporary portion (cycling segment) that cycles through anagen (active

growth), catagen (apoptosis-driven retraction), and telogen (a resting period) (Figs. 1A and 2A) (11, 12). Signals emanating from the underlying dermal papilla (DP) induce activation of the stem cells in the hair germ (a cluster of cells located at the bottom of bulge) and the bulge (13). During anagen, the distance between the bulge and DP increases, which modifies the activation state of the stem cells (Fig. 2A).

Cotsarelis and colleagues were the first to identify label-retaining cells (LRCs) in the bulge region (7). However, because of the inability to isolate live 5-bromo-2'-deoxyuridine-positive (BrdU<sup>+</sup>) LRCs, they could not determine whether bulge LRCs were stem cells. Fuchs and colleagues developed an elegant method to isolate live LRCs by generating a doxycycline-controlled histone2B-green fluorescent protein (H2B-GFP) mouse line (14). Expression of H2B-GFP was seen in almost all of the epidermal (including stem) cells without adding doxycycline. When doxycycline



**Fig. 1.** Stem cell locations in hair follicle, gut, and bone marrow. (A) Hair follicle structure with quiescent (bulge) and active (hair germ) stem/progenitor cells. Bulge area typically maintains quiescent stem cells, whereas DP provides stimulatory signals. Only during development and under injury condition, bulge stem cells give rise to stem cells in epidermis. (B) Intestinal crypt structure with quiescent (+4) and active CBC (Lgr5<sup>+</sup>) stem cells, as well as TA and mesenchymal cells. (C) Quiescent HSCs located in the endosteal region where osteoblastic lining, endothelial, CAR, and other cells form the endosteal region and active HSCs located in the central marrow region, which lacks osteoblastic cells.

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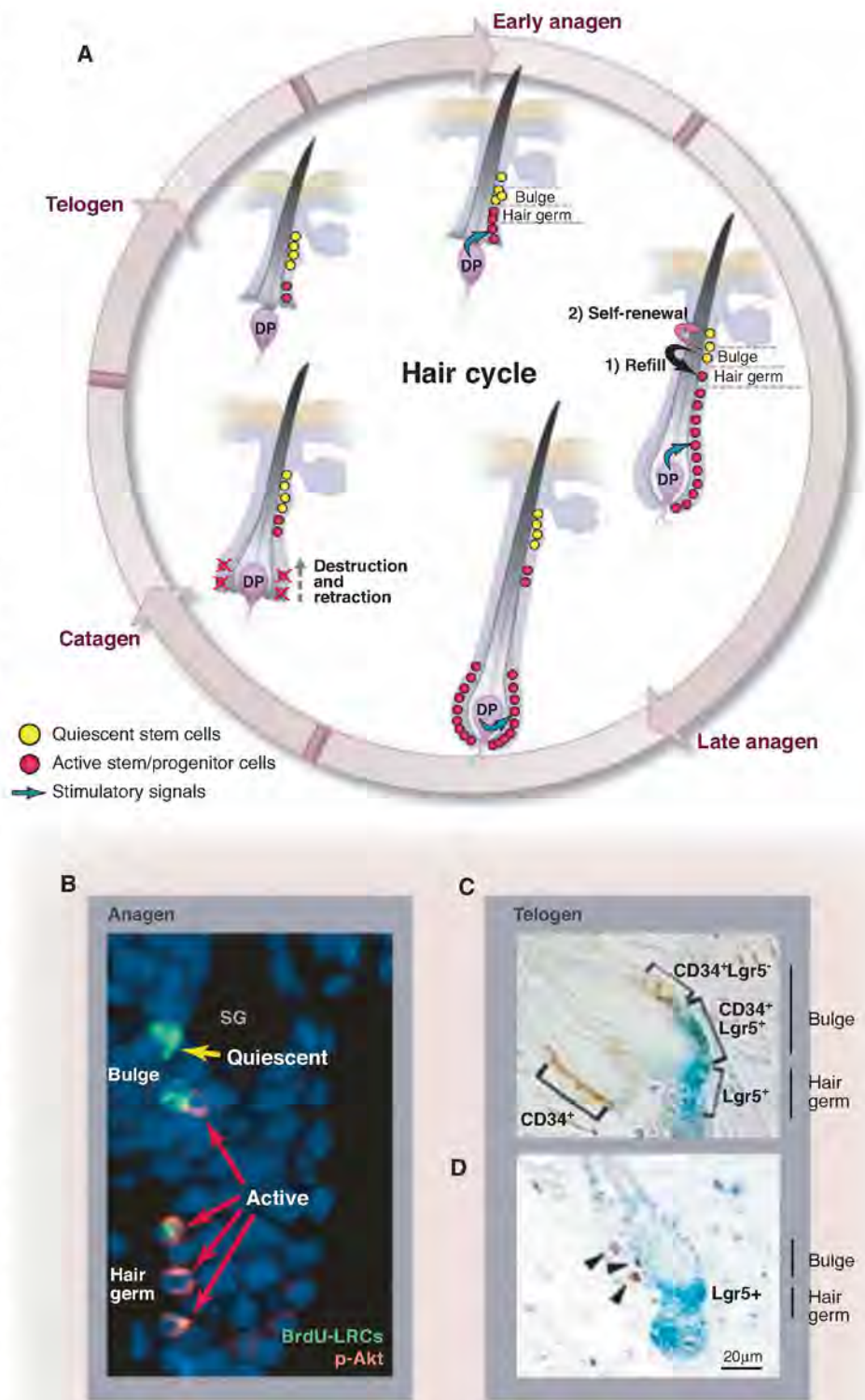
was administered to young mice for 1 month, most cells diluted out the H2B-GFP label by division during the chasing process. Some cells in the bulge, however, retained H2B-GFP and were thus identified as GFP-expressing LRCs. The majority of these H2B-GFP LRCs expressed the stem cell marker CD34 (14). Sorted and cultured CD34<sup>+</sup> bulge cells from a keratin14-GFP transgenic mouse were transplanted and shown to generate the entire hair follicle (15). Taken together, these studies demonstrate that LRCs (here identified as CD34<sup>+</sup> and KI14<sup>+</sup>) in the hair follicle represent functional stem cells (15).

Although many bulge cells can be activated from the quiescent state to enter the cell cycle during anagen (15), some LRCs retain labels over many months, suggesting that they exist in a quiescent state and are not actively involved in hair regeneration (16) (Fig. 2B). These observations suggest that bulge cells are heterogeneous in terms of cycling state (17).

Bulge stem cells were recently found not to directly generate TA cells but rather give rise to an intermediate stem cell population located at the hair germ. These hair germ cells in turn produced the TA cells, which further differentiated into the hair shaft in the hair matrix (13, 18). Leucine-rich repeat-containing heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptor (Lgr) 5 is expressed in the bulge region and the hair germ during late telogen/early anagen as well as in the hair matrix during late anagen (Fig. 2C) (19). In the bulge, the CD34<sup>+</sup> compartment consists of both label-retaining Lgr5<sup>+</sup> cells and active Lgr5<sup>+</sup> cells located side by side (Fig. 2C). This is consistent with the detection of Akt-phosphorylated (activated) LRCs in the lower bulge region and hair germ during anagen (Fig. 2B) (20). Indeed, the Lgr5<sup>+</sup> cells seem to be the first to proliferate upon induction of anagen, and some Lgr5<sup>+</sup> cells persist through several hair cycles (19) as revealed by in vivo lineage tracing. Thus, the location of Lgr5<sup>+</sup> cells in the lower bulge and extending into the hair germ is consistent with the recent finding that activation of bulge and hair germ stem cells occurs in two steps (13). Activation of bulge cells follows activation of hair germ cells, indicating that the bulge cells are replenishing hair germ cells that have entered the hair matrix to support hair growth (Fig. 2A) (18). On the basis of these observations, the hair follicle appears to contain both quiescent and active stem cell populations in separate yet adjacent locations.

## Gut

In the intestinal epithelium, stem cells and their short-lived TA cells reside in crypts (Fig. 1B). Cells exiting the crypts and entering the villi terminally differentiate into enterocytes, goblet cells, or enteroendocrine cells. Paneth cells escape this course by migrating to crypt bottoms. The intestinal epithelium is renewed about every 5 days (21). Potten and others have proposed



**Fig. 2.** DP position determines state of stem/progenitor cells in hair follicle. **(A)** Hair cycle. At the start of anagen, DP is proximal to hair germ and bulge. Stem/progenitor cells in hair germ (a structure below the bulge) are activated by DP first, whereas stem cells remain quiescent in bulge. When hair germ cells enter the hair matrix, then stem cells in bulge are activated to replenish lost hair germ cells. During anagen, active hair germ stem cells give rise to TA cells, which in turn support hair growth. DP is pushed down from bulge because of fast expansion of progenitor cells. In catagen, DP retracts toward bulge. **(B)** Co-staining of BrdU with phosphorylated-Akt shows relationship between quiescent stem cells (yellow arrow) and active stem cells (red arrows) in bulge and hair germ. [Reprinted by permission from John Wiley and Sons, Incorporated, AlphaMed Press, p. 2834 of (20).] **(C and D)** CD34<sup>+</sup>Lgr5<sup>−</sup> and CD34<sup>+</sup>Lgr5<sup>+</sup> stem cells, respectively, located inside and next to bulge area (C), which is consistent with Lgr5<sup>+</sup> stem cells located adjacent to LRCs (D). [Reprinted by permission from Macmillan Publishers, Limited, pp. 1292 and 1293 of (19).]



that LRCs (on average 2 to 4 out of 16 cells in a ring) at the +4 position located above the Paneth cells (Fig. 1B) represent stem cells (8). A recent study using *in vivo* lineage tracing has shown that cells expressing Bmi1 predominantly mark +4 position and are able to give rise to all four epithelial lineages (22). Although Bmi1<sup>+</sup> cells were suggested to be slowly cycling, it is currently unknown whether they are LRCs (i.e., quiescent). Some cells at the +4 cell position that express the potential stem cells marker Musashi-1 are not sensitive to inactivation of the cell cycle protein CDC25, suggesting they have quiescent features (23).

In an alternative model, crypt-based columnar cells (CBCs) specifically expressing Lgr5 located at crypt bottoms among Paneth cells represent intestinal stem cells (ISCs) (Fig. 1B) (24). Lineage tracing has shown that Lgr5<sup>+</sup> cells represent a long-lived and cycling multipotent stem cell population (25). A single Lgr5<sup>+</sup> stem cell can form a long-lived, self-renewing "mini-gut" in culture (26). Lgr5<sup>+</sup> stem cells are distinct from +4 LRCs in that Lgr5 stem cells do not retain DNA labels and are sensitive to CDC25 inactivation, supporting their proliferating feature (23). Thus, the intestine contains a quiescent stem cell population at the +4 position and a cycling Lgr5 stem cell population among the Paneth cells. Future studies are needed to determine the relationship between these cell populations.

### Bone Marrow

Studies of hematopoietic stem cells (HSCs) have shaped our thinking on most basic features of mammalian stem cells (1). HSCs give rise to a hierarchically organized set of progenitors for erythroid, myeloid, lymphoid, and megakaryocyte lineages (1). The mainstay of HSC analysis has been marker-based cell sorting followed by transplantation in lethally irradiated recipients. This assay tests self-renewal as well as multilineage potential.

Most primitive HSCs are quiescent (27). Using DNA label BrdU and H2B-GFP incorporation respectively (14), LRCs were found to be predominantly located in the endosteum (Fig. 1C) (28) and subsequently confirmed to be a primitive HSC population (29, 30). The quiescent HSCs have superior long-term reconstitution potential; however, HSCs in this population cycle only once every 145 days on average and thus may not provide ongoing support for production of billions of blood cells, although they can be activated for this function under injury conditions (29). Other studies based on the DNA label BrdU and H2B-GFP incorporation have suggested that the majority of murine HSCs undergo more frequent cycling (29, 31–33). These seemingly disparate findings may be reconciled by postulating the existence of two subpopulations of HSCs, one being long-term quiescent ("reserved") and the other being more actively cycling ("primed") (Fig. 1C) (29, 33, 34). Primed

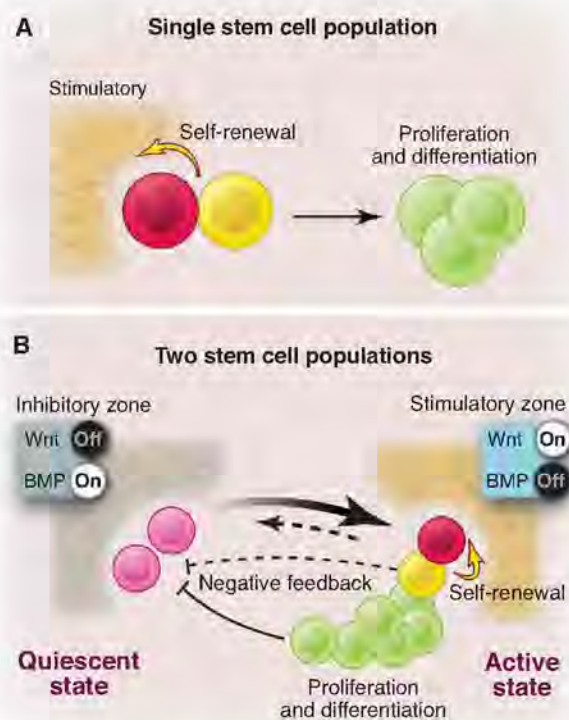
HSCs may be the workhorse that supports the daily production of billions of blood cells, whereas reserved HSCs function as a "backup" (to replenish lost active stem cells under homeostasis and particularly in response to injury or pathological challenge) (29, 33).

### Stem Cell Zones and Associated Microenvironmental Signals

The observations described above do not easily conform to a model in which a single stem cell population maintains tissue self-renewal (Fig.

migrating upward from the crypt bottom and passing +4 may potentially provide a negative feedback to the quiescent stem cells. Thus, loss of CBCs or their progeny may trigger the activation of quiescent stem cells. Different assays may be biased in revealing one or the other population of stem cells: transplantation assays document "stem cell potential" and may favorably detect the reserved population, particularly in the serial transplantation assays, whereas *in vivo* lineage tracing assays visualize the primed population.

How can these two very different states of stem cell subpopulations be maintained at separate but adjoining locations? The nature of the locations of stem cell subpopulations in hair follicle and intestine suggests that there are segregated zones that determine the active or quiescent state of the stem cells. Individual stem cell niches are maintained by the secretion of specific proteins. For example, the hair follicle bulge constitutes a canonical Wnt-off/bone morphogenetic protein (BMP)-on microenvironment through the expression of secreted Dkk-1, sFRP, Wif, and BMP (14). In contrast, the DP secretes Wnt signals and noggin, an antagonist of BMP signaling (11) (Fig. 4A). Similarly, cycling Lgr5<sup>+</sup> stem cells at the crypt bottom in intestine are in a microenvironment with high Wnt activity (10), whereas BMP signaling is inhibited by noggin and gremlin produced by submucosal tissue below the crypts (37, 38). Stem cells at +4 positions encounter BMP4 and the Wnt inhibitor sFRP5 (37, 39) (Fig. 4B). In bone marrow, endosteal region [composed of osteoblastic, vascular (40), and CXCL12 abundant reticular (CAR) cells (41), as well as osteoclasts (42)] and central marrow region (lacking osteoblastic signals) have been shown to form inhibitory and stimulatory zones respectively

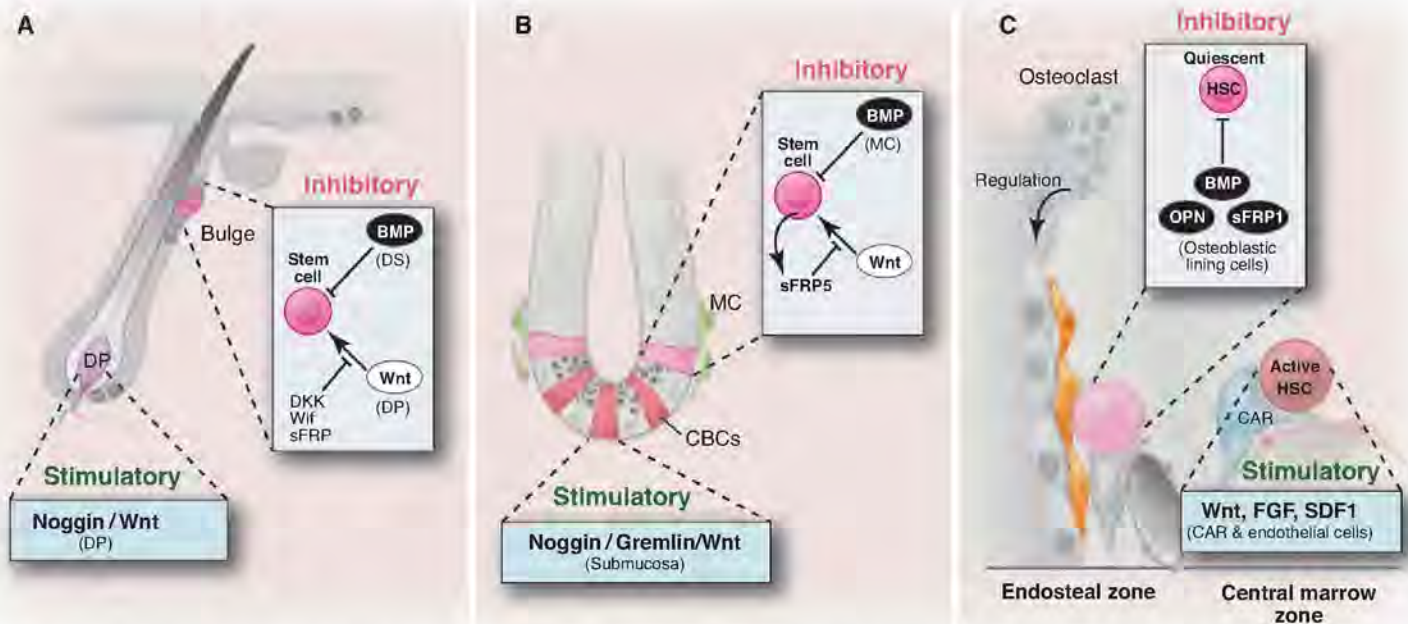


**Fig. 3.** Two models for stem cell-based tissue self-renewal and regeneration. (A) Prevailing model of a single stem cell population located in the niche. Asymmetric division is the key mechanism to maintain balance between self-renewal and differentiation. (B) Proposed alternative and complementary model: co-existing quiescent and active stem cell populations located in adjacent zones with corresponding inhibitory and stimulatory signals. Quiescent stem cells replace damaged active stem cells (heavy arrow). Conversely, active stem cells may replace lost quiescent stem cells (light arrow). A negative feedback from either active stem cells (dashed line) or their progeny (solid line) may contribute to prevention of quiescent stem cells from activation.

3A). An alternative "zoned" model, in which quiescent and active stem cells coexist within the same tissue, may better explain these observations (35). In the zoned stem cell model, active stem cells are the primed subpopulation that account for most of the replenishment of corresponding tissues, whereas quiescent stem cells function as a backup or reserved subpopulation. This reserved population can be activated either by a stochastic mechanism (36) or by feedback upon loss of active stem cells or extensive tissue damage (Fig. 3B). For example, the cycling CBC stem cells and/or their progeny

ly (43, 44) (Fig. 4C). Intriguingly, HSCs in aged mice localized more distantly to the endosteum than in young mice, supporting the existence of two zones in bone marrow, with the central marrow zone favoring proliferation of HSCs (45). This correlates with the increasing number but decreasing function of aged HSCs (46). Under homeostatic conditions, BMPs (28), Osteopontin (47), Wnt signaling inhibitors sFRP1 (48), as well as potentially noncanonical Wnts are expressed in the endosteal zone, providing an inhibitory microenvironment. Fibroblast growth factors (FGFs) and canonical Wnts expressed from endo-





**Fig. 4.** Zoned stem cell population and the associated microenvironmental signals. **(A)** Bulge area typically provides Wnt-off and BMP-on signals thus maintaining quiescent stem cells, whereas DP provides stimulatory (Wnt-on and BMP-off) signals. **(B)** Similarly, in intestinal crypt (+4) ISCs are maintained by Wnt-off and BMP-on signals. In contrast, CBC (Lgr5<sup>+</sup>) stem cells

are exposed to Wnt-on but BMP-off signals. **(C)** Quiescent HSCs located in the endosteal zone where osteoblastic lining cells provide dominant inhibitory signals including BMP, OPN, and sFRP1. In contrast, HSCs located in the central marrow zone are stimulated by endothelial, megakaryocyte, and CAR cells secreting Wnt, FGF, and SDF1.

thelial, megakaryocytes, and CAR cells may have a dominant and stimulatory influence on the central marrow zone under homeostatic conditions (33, 49, 50) (Fig. 4C). The locations of these cells and the associated signals might change, however, under stressed conditions (43).

### Functional Significance of Zoned Stem Cell Subpopulations

What could be the advantage of maintaining zoned subpopulations of stem cells? We speculate that increasing life span and body size during evolution exerted selective pressure to increase the longevity and output of adult stem cell pools, particularly in the rapidly regenerating tissues, without increasing the risk for (tumorigenic) mutations. The adaptation of reserved quiescent stem cell pools may provide a mechanism to increase the longevity of adult stem cells through population replacement (Fig. 3B). Here, population replacement describes a mechanism by which one population of cells is replaced by another and should be distinguished from the classic mechanism of self-renewal at the individual cell level. This is exemplified by lost stem cells in hair germ being replenished by stem cells located in the bulge during each hair cycle (13). Normally, quiescent stem cells would be expected to replace damaged active stem cells (Fig. 3B). This would prevent the active stem cell pool from becoming exhausted and protect against accumulating potentially tumorigenic mutations during DNA replication. Conversely, active stem cells would possibly have the capacity to replace lost or damaged quiescent

stem cells under very special circumstances (Fig. 3B). The combination of these reciprocal backup systems would provide a robust mechanism to ensure a high rate of physiological self-renewal as well as flexible damage repair, after which the original hierarchy could be reestablished.

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# Role of ABA and ABI3 in Desiccation Tolerance

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To survive on land, the earliest land plants had to develop mechanisms to tolerate desiccation. Modern vascular plants possess an array of morphological features to retain water (such as conductive tissues, cuticle, and stomata) and have retained desiccation tolerance in only a few specialized structures (e.g., seeds). Present-day bryophytes (mosses), in contrast, lack water transport and retention tissues, presumably like early land plants. As a result, their vegetative state is at equilibrium with the surrounding air, creating a water-deficit condition that most angiosperms could not tolerate (1). Phylogenetic analyses suggest that desiccation tolerance in vegetative tissue of bryophytes was lost in the first vascular plants (2). Here, we evaluate whether desiccation tolerance in angiosperm seeds and in vegetative tissues of the moss *Physcomitrella patens* use similar regulatory pathways.

The phytohormone abscisic acid (ABA) protects seeds during water stress by activating genes through transcription factors such as *ABSCISIC ACID INSENSITIVE 3* (*ABI3*) (3).

ABA is also found in nonseed plants such as algae and *P. patens* (4) and uses similar signaling pathways. For example, a wheat ABA-responsive promoter can be activated by ABA in cells of *P. patens* (5), and one of three homologs of *ABI3* found in *P. patens* partially complements the *Arabidopsis abi3-6* mutant (6).

Untreated wild-type (WT) filaments of *P. patens* can survive up to 92% water loss (7) but cannot recover from complete desiccation (Fig. 1A). We generated two independent lines (*Δabi3-1* and *Δabi3-2*) in which all three *P.*

*patens ABI3* genes (A, B, and C) were deleted by using sequential gene targeting (fig. S1) (8, 9). WT lines survived if incubated with ABA (10 to 100 μM) for 24 hours before desiccation, whereas two *Δabi3* lines did not survive, even at 100 μM ABA (Fig. 1A). The *Δabi3* lines were also not responsive to an ABA-responsive promoter from moss (*PpLEA1a-GUS*), whereas WT exhibited an increase (fig. S2). Expression of 22 ABA up-regulated genes from WT *P. patens* (that are presumably required for tolerance) were compared with those of *Δabi3* at 24 hours after ABA treatment, 24 hours after drying, and 5 min and 15 min after rehydration (Fig. 1B). Without *PpABI3*, only a few transcripts had reduced expression after ABA treatment and drying, whereas the others maintained their expression. The loss of *PpABI3* had little effect on this subset of ABA up-regulated genes before rehydration. However, all 22 genes assayed at 5 and 15 min after rehydration showed drastically reduced transcripts or none at all in the *Δabi3-1* line when compared with WT (Fig. 1B). For successful recovery from desiccation, *PpABI3* appears to be essential for the maintenance, either by synthesis or stabilization, of those transcripts induced during the ABA pretreatment that are critical for tolerance.

We conclude that both ABA and *ABI3* are required for *P. patens* vegetative tissue to survive desiccation. Because the *P. patens* genome lacks the transcription factors *FUS3* and *LEC2* (10) that are required for seed maturation like *ABI3* (3), the role of *ABI3* in this nonseed plant appears to be directly in desiccation tolerance,

primarily in the recovery stage. Our working hypothesis is that gene regulatory pathways that include both ABA and *ABI3* originally evolved for cellular protection from water deficits but independently have been used to provide desiccation tolerance in vegetative tissues of bryophytes and in angiosperm seeds.

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## Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5965/546/DC1

Materials and Methods

Figs. S1 and S2

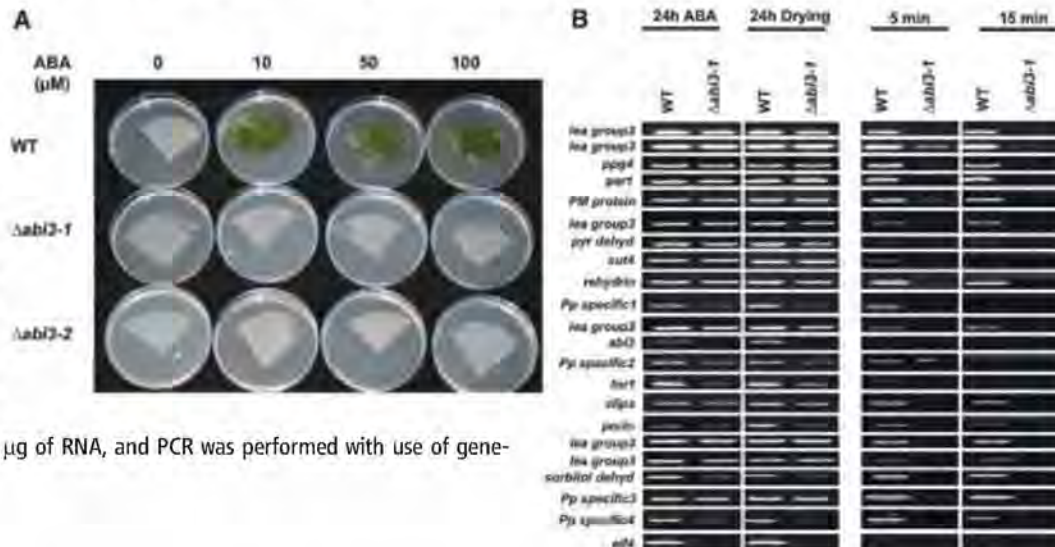
Table S1

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**Fig. 1.** (A) ABA and *PpABI3* are required for desiccation tolerance. Tissues from 6-day-old WT, *Δabi3-1*, and *Δabi3-2* were treated with ABA (0, 10, 50, and 100 μM) for 24 hours. Tissues were dried for 24 hours, rehydrated with sterile distilled water, and incubated for 2 weeks. (B) Reverse transcription polymerase chain reaction (RT-PCR) analysis of ABA-induced transcripts in WT and *Δabi3-1* during ABA treatment, drying, and rehydration. RNA was extracted from 6-day-old tissues 24 hours after ABA treatment, 24 hours after drying, and 5 and 15 min after rehydration in basal medium. cDNA was synthesized with use of 2 μg of RNA, and PCR was performed with use of gene-specific primers (table S1).





# Local and Long-Range Reciprocal Regulation of cAMP and cGMP in Axon/Dendrite Formation

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Cytosolic cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) often mediate antagonistic cellular actions of extracellular factors, from the regulation of ion channels to cell volume control and axon guidance. We found that localized cAMP and cGMP activities in undifferentiated neurites of cultured hippocampal neurons promote and suppress axon formation, respectively, and exert opposite effects on dendrite formation. Fluorescence resonance energy transfer imaging showed that alterations of the amount of cAMP resulted in opposite changes in the amount of cGMP, and vice versa, through the activation of specific phosphodiesterases and protein kinases. Local elevation of cAMP in one neurite resulted in cAMP reduction in all other neurites of the same neuron. Thus, local and long-range reciprocal regulation of cAMP and cGMP together ensures coordinated development of one axon and multiple dendrites.

The polarization of postmitotic neurons involves the differentiation of a single axon and multiple dendrites. The process of neuronal polarization begins with the specification of the axon/dendrite identity of undifferentiated neurites, followed by selective localization of molecules that are responsible for differential growth and specific functions of the axon and dendrites (1, 2). The initial axon/dendrite specification may result from cytoplasmic asymmetry caused by the last mitotic division of the neural progenitor cell (3), the stochastic fluctuation of cytoplasmic determinants (1, 2, 4, 5), or the action of extracellular polarizing factors (6–12). In cultured hippocampal neurons, neuronal polarization appears to begin with selective accumulation/activation of key components into one undifferentiated neurite (1, 8, 9, 12–19) that triggers its rapid growth and axon differentiation. To ensure the formation of one axon and multiple dendrites, axon initiation at one neurite must be accompanied by long-range signals that suppress axon formation or promote dendrite formation in other neurites. We investigated the role of two second messengers, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), in mediating the co-

ordinated differentiation of one axon and multiple dendrites.

**Antagonistic axon/dendrite initiation by local cAMP/cGMP activity.** Dissociated embryonic hippocampal neurons undergo spontaneous polarization on a uniform culture substratum, from a morphologically symmetric cell after plating to a polarized neuron exhibiting a single axon and multiple dendrites within a few days (20, 21). First, we examined the effect of localized cAMP and cGMP activities in axon/dendrite initiation by plating these neurons on substrates coated with stripes of membrane-permeant fluorescent analogs of cAMP or cGMP [F-cAMP or F-cGMP; see supporting online material (SOM)]. The neurons were imaged at 12 hours after plating and were immunostained after further incubation of 48 hours with the axon marker Smi-312 and the somatodendritic marker MAP2. At 12 hours, cells exhibited several short neurites of similar lengths (Fig. 1, A and B). By 60 hours, axons were found on the F-cAMP stripe or off the F-cGMP stripe, whereas dendrites were mostly found off the F-cAMP stripe or on the F-cGMP stripe (Fig. 1, A and B). We analyzed the distribution of axon/dendrite initiation sites on the soma at 48 to 60 hours after plating, when neurons had completed the polarization process, for all polarized cells with the soma located at the stripe boundary (Fig. 1Ca). This retrospective analysis of axon/dendrite initiation was possible because neurite initiation sites on the soma did not move during neuronal polarization (Fig. 1, A and B). The distribution of the axon initiation site showed preference for initiation on the F-cAMP stripe and off the F-cGMP stripe, but no preference for stripes coated with bovine serum albumin (Fig. 1Cb). The distribution of dendrite initiation sites showed opposite pref-

erence (Fig. 1Cb). Analysis of the preference index  $\{PI = [(\% \text{ on stripe}) - (\% \text{ off stripe})]/100\}$  showed that localized cAMP and cGMP activities were sufficient to induce preferential initiation of axons and dendrites, respectively (Fig. 1D). These effects are distinct from the known effects of cAMP and cGMP on the guidance of growth cones (22, 23) that were also revealed by the axon/dendrite pathfinding at the stripe boundary (Fig. 1, A and B).

Next, we plated these neurons on a substrate striped with either the adenylyl cyclase (AC) inhibitor SQ-22536 or the protein kinase A (PKA) inhibitor KT5720 or Rp-8-Br-cAMPS, to reduce locally basal cAMP/PKA activity. For neurons with the soma located at the stripe boundary, axons were mostly initiated off the stripes (Fig. 1, Cb and D), similar to that found on the F-cGMP-striped substrate. Conversely, when the neurons were plated on a substrate striped with the soluble guanylate cyclase (sGC) inhibitor ODQ or the protein kinase G (PKG) inhibitor KT5823 or Rp-8-pCPT-cGMPS, axons were initiated mostly on the stripe (Fig. 1, Cb and D), similar to that found for F-cAMP stripes. There was also a preference for the dendrite to be initiated off the ODQ/KT5823/Rp-8-pCPT-cGMPS stripes, but on the SQ-22536/KT5720/Rp-8-Br-cAMPS stripes (Fig. 1, Cb and D). Thus, asymmetry in the basal cAMP or cGMP activity is sufficient to trigger axon/dendrite polarization, and cAMP and cGMP activities exert antagonistic actions on this process.

**Reciprocal regulation between cAMP and cGMP.** Antagonistic cellular actions mediated by cAMP and cGMP (23–28) could result from opposing actions of cAMP and cGMP pathways on common cellular targets. However, crosstalk between these two pathways could also occur by reciprocal regulation between cAMP and cGMP, as suggested by findings from non-neuronal systems (28, 29). We used genetically encoded fluorescence resonance energy transfer (FRET) reporters to examine directly the reciprocal regulation between cAMP and cGMP in cultured hippocampal neurons. The neurons were transfected 2 hours after cell plating with a construct encoding one of the three FRET reporters: indicator of cAMP using Epac (ICUE) (30), cGMP energy transfer sensor derived from PDE5A (cGES-DE5) (31), or A-kinase activity reporter (AKAR) (32) (SOM text), which were designed to monitor the amount of cAMP, cGMP, and PKA activity, respectively. All FRET measurements were made 10 to 16 hours after cell plating, before axon/dendrite differentiation (20, 21). Bath application of the membrane-permeant cAMP analog Sp-8-Br-cAMPS (20  $\mu$ M) or the AC activator forskolin (20  $\mu$ M) resulted in a global increase of cAMP and PKA signals in ICUE- and AKAR-expressing cells, respectively, as measured by the increase in the ratio of yellow fluorescent protein (YFP) to cyan fluorescent protein (CFP) fluorescence at the neurite (Fig. 2,

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A, B, D, E, and G). In contrast, the same treatment induced a reduction of cGMP in cells expressing cGES-DE5 (Fig. 2, C, F and G). Conversely, the membrane-permeant cGMP analog 8-pCPT-cGMP (20  $\mu$ M) or the nitric oxide donor (DEA NONOate, 200  $\mu$ M), which is known to activate sGC, increased cGMP (Fig. 2, C, F and G) and reduced cAMP and PKA activity (Fig. 2, B, D, E and G).

The reciprocal regulation was also observed when the basal level of either cAMP or cGMP

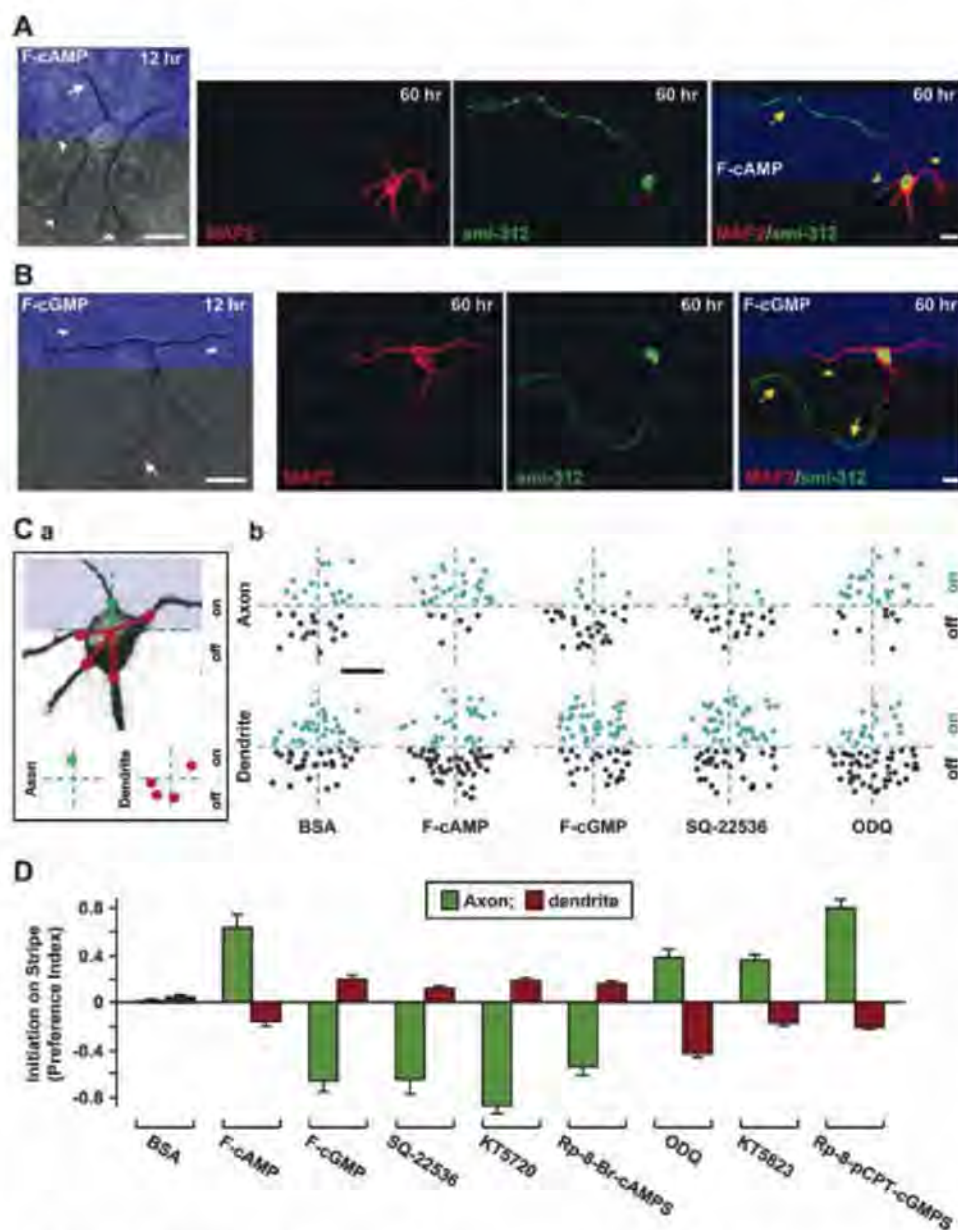
was modulated by altering its synthesis or degradation. Bath application of the AC inhibitor SQ-22536 (10  $\mu$ M) resulted in not only the expected reduction of cAMP and PKA activity, but also in cGMP elevation (Fig. 2G). Treatment with the sGC inhibitor ODQ (1  $\mu$ M) yielded opposite effects: reduction of cGMP and elevation of cAMP and PKA activity (Fig. 2G). Furthermore, the nonspecific phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (IBMX, 50  $\mu$ M) elevated both the basal cAMP/PKA

and cGMP levels, whereas the cAMP-specific PDE4 inhibitor rolipram (1  $\mu$ M) caused an expected increased cAMP/PKA level and a slight cGMP reduction (Fig. 2G). A peptide-based assay also confirmed that IBMX could elevate PKA activity in hippocampal cultures (fig. S3Cb), and that rolipram treatment or transfection with PDE4D small interfering RNAs (siRNAs) caused similar PKA elevation in cultured HEK-293T cells (fig. S1A). Thus, reciprocal regulation between cAMP and cGMP is constitutively present in these developing neurons, allowing adjustment of the basal level of these cyclic nucleotides bi-directionally. The antagonistic actions of cAMP/cGMP on axon/dendrite differentiation described above (Fig. 1) could be attributed directly to the reciprocal regulation between these two cyclic nucleotides.

#### Mechanisms underlying reciprocal regulation.

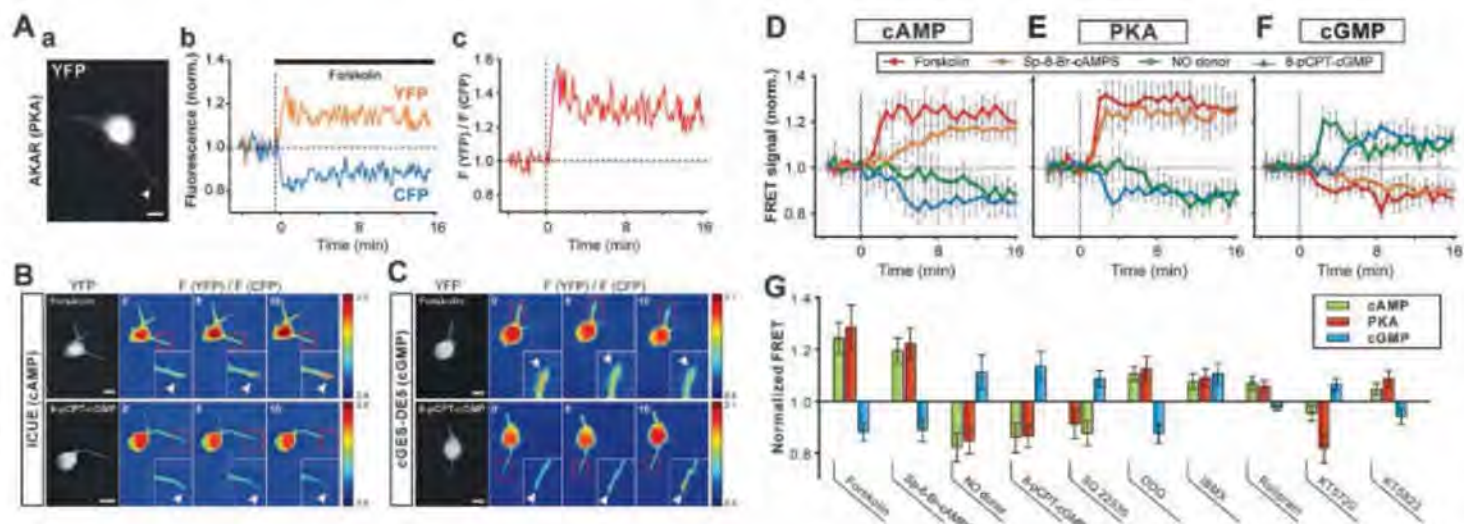
In various non-neuronal systems, cGMP-induced activation of PDEs inhibits cAMP signaling by increasing cAMP hydrolysis (29, 33). Measurements with FRET reporters in cultured neurons showed that, after pre-incubation with IBMX, subsequent addition of NO-donor (or 8-pCPT-cGMP) and forskolin failed to induce reciprocal down-regulation of cAMP/PKA activity (Fig. 3, Aa and Ab) and cGMP (Fig. 3Ac), respectively, demonstrating the involvement of PDEs in this reciprocal regulation. Pre-incubation with rolipram, which by itself elevated the basal level of cAMP/PKA activity (Fig. 2G), abolished the 8-pCPT-cGMP-induced reduction of the PKA activity (Fig. 3Ad). The rolipram effect was specific, because the PDE2-specific inhibitor BAY 60-7550 had no effect (Fig. 3Ae), and it was not attributable to saturated cAMP elevation caused by rolipram, because further cAMP elevation could be achieved by adding forskolin to rolipram-treated cells (Fig. 3Af). Similarly, for the crosstalk from cAMP to cGMP, the forskolin-induced cGMP reduction could be abolished by the PDE3 inhibitor cilostamide (300 nM) (Fig. 3Ag) and was significantly diminished by the inhibitor of cGMP-specific PDE5 MY-5445 (30  $\mu$ M) (Fig. 3Ah). Furthermore, although an NO donor greatly reduced the forskolin-induced PKA activity (Fig. 3Ba), it had no significant effect on the PKA activity induced by the nonhydrolyzable cAMP analog Sp-8-Br-cAMPS (Fig. 3Bb). Similarly, forskolin suppressed NO donor-induced cGMP elevation (Fig. 3Bc) but had no significant effect on the cGMP signal induced by the nonhydrolyzable cGMP analog 8-pCPT-cGMP (Fig. 3Bd).

In non-neuronal systems (29, 33), PDE activities can be modulated by PKA/PKG-dependent phosphorylation. We found that forskolin-induced cGMP reduction was prevented by KT5720 (200 nM, Fig. 3Ai), and the reduction of PKA activity that was caused by 8-pCPT-cGMP was prevented by the PKG inhibitor KT5823 (200 nM, Fig. 3Aj). Thus, whereas reciprocal regulation between cAMP and cGMP is modulated by specific PDEs, PKA/PKG activation might



**Fig. 1.** Antagonistic effects of cAMP and cGMP on axon/dendrite initiation. (A and B) Images of cultured hippocampal neurons on substrates coated with stripes (blue) of F-cAMP or F-cGMP at 12 and 60 hours after cell plating and immunostained (at 60 hours) for axons and dendrites with smi-312 and MAP2 antibodies, respectively. White arrows and arrowheads: neurites that later became axons and dendrites, respectively. Yellow symbols: axon/dendrite turning at stripe boundary. Scale bars, 10  $\mu$ m. (C) Preferential axon/dendrite initiation. (a) Diagram depicting the distribution of axon/dendrite initiation site (circles) relative to the center of the stripe boundary intersecting the soma. (b) Distribution of initiation sites on-stripe (blue) or off-stripe (black), for axons (30 cells) and all dendrites (10 cells) initiated on various striped substrates. Scale bar, 5  $\mu$ m. (D) Preferential index for axon/dendrite initiation, shown as average  $\pm$  SD [ $n = 3$  to 5 cultures, 150 to 200 cells each,  $P < 0.001$ , two-tailed  $t$  test or Kolmogorov-Smirnov test, compared to bovine serum albumin (BSA)].

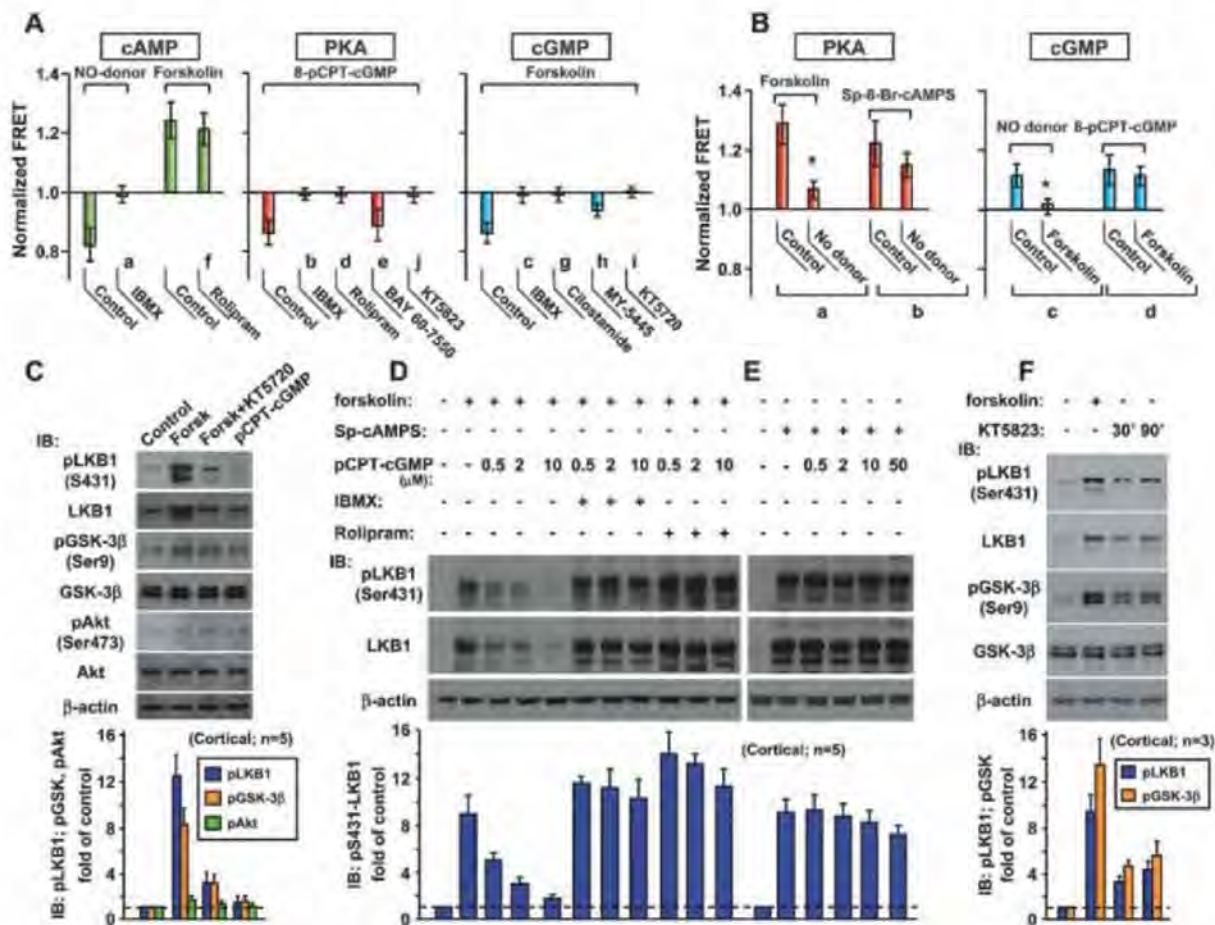




**Fig. 2.** Reciprocal regulation between cAMP and cGMP revealed by FRET imaging. **(A)** Forskolin-induced FRET signal for PKA activity. **(a)** YFP image of a hippocampal neuron expressing AKAR at 16 hours. Scale bar, 10  $\mu$ m. **(b)** Forskolin-induced changes in YFP and CFP fluorescence at a neurite tip (red square in **a**), normalized by the average value before forskolin application. **(c)** Ratio of normalized YFP fluorescence [F(YFP)] to CFP fluorescence [F(CFP)], representing the FRET signal. **(B and C)** FRET signals observed at the neurite tip of 16-hours neurons expressing ICUE or cGES-DE5. The black and white images show YFP fluorescence and the color show FRET signals at different times (in min) after forskolin or 8-pCPT-cGMP ap-

plication, coded in pseudocolors (scale bar on the right). Inset: Higher magnification images of FRET signals at the neurite tip. Scale bar, 10  $\mu$ m. **(D to F)** FRET signal (at neurite tips) for all neurons expressing the ICUE [(D), cAMP], AKAR [(E), PKA] or cGES-DE5 [(F), cGMP], induced by the compound as indicated, averaged over 40-s bins and normalized by the mean control value before drug application ( $\pm$ SEM,  $n = 5$  to 8 cells each, 1 or 2 per cell). **(G)** Reciprocal regulation between cAMP and cGMP and the role of PDEs and PKA/PKG. Data represent average FRET signals similar to that in (D) to (F) for cAMP, PKA, or cGMP at 10 to 20 min after drug application. Error bars indicate  $\pm$ SEM,  $n = 5$  to 8 cells each, 1 or 2 per cell.

**Fig. 3.** The role of PDEs and PKA/PKG in reciprocal cAMP/cGMP regulation and phosphorylation of axon determinants LKB1 and GSK-3 $\beta$ . **(A and B)** Average FRET signals for cAMP, PKA, and cGMP at 10 to 20 min after bath application of the compound indicated above, in the presence of the drug indicated below ( $\pm$ SEM, 5 to 8 cells each, 1 or 2 per cell). In **(A)** and **(B)**, data significantly different from control are marked by an asterisk ( $P < 0.05$ , Mann-Whitney U test). **(C)** Immunoblot analysis of cAMP-induced phosphorylation of LKB1, GSK-3 $\beta$ , and Akt on total cell lysates of 5-day cultures of cortical neurons, using antibodies specific to the phosphorylation site or the protein. Forsk., forskolin. Histograms show the average phosphorylation level ( $\pm$ SD,  $n = 5$ ), normalized to  $\beta$ -actin and shown as fold of control. **(D and E)** Antagonistic action of cGMP activity on cAMP-induced LKB1 phosphorylation is mediated by cAMP-specific PDEs. Immunoblot analysis as in **(C)**. Cells were treated with forskolin (**D**) or Sp-8-Br-cAMPS (**E**), either alone or



in combination with increasing concentrations of 8-pCPT-cGMP, and in the absence or presence of IBMX or rolipram. **(F)** Inhibition of PKG by KT5823 resulted in LKB1 and GSK-3 $\beta$  phosphorylation. Immunoblot analysis was as in **(C)**.



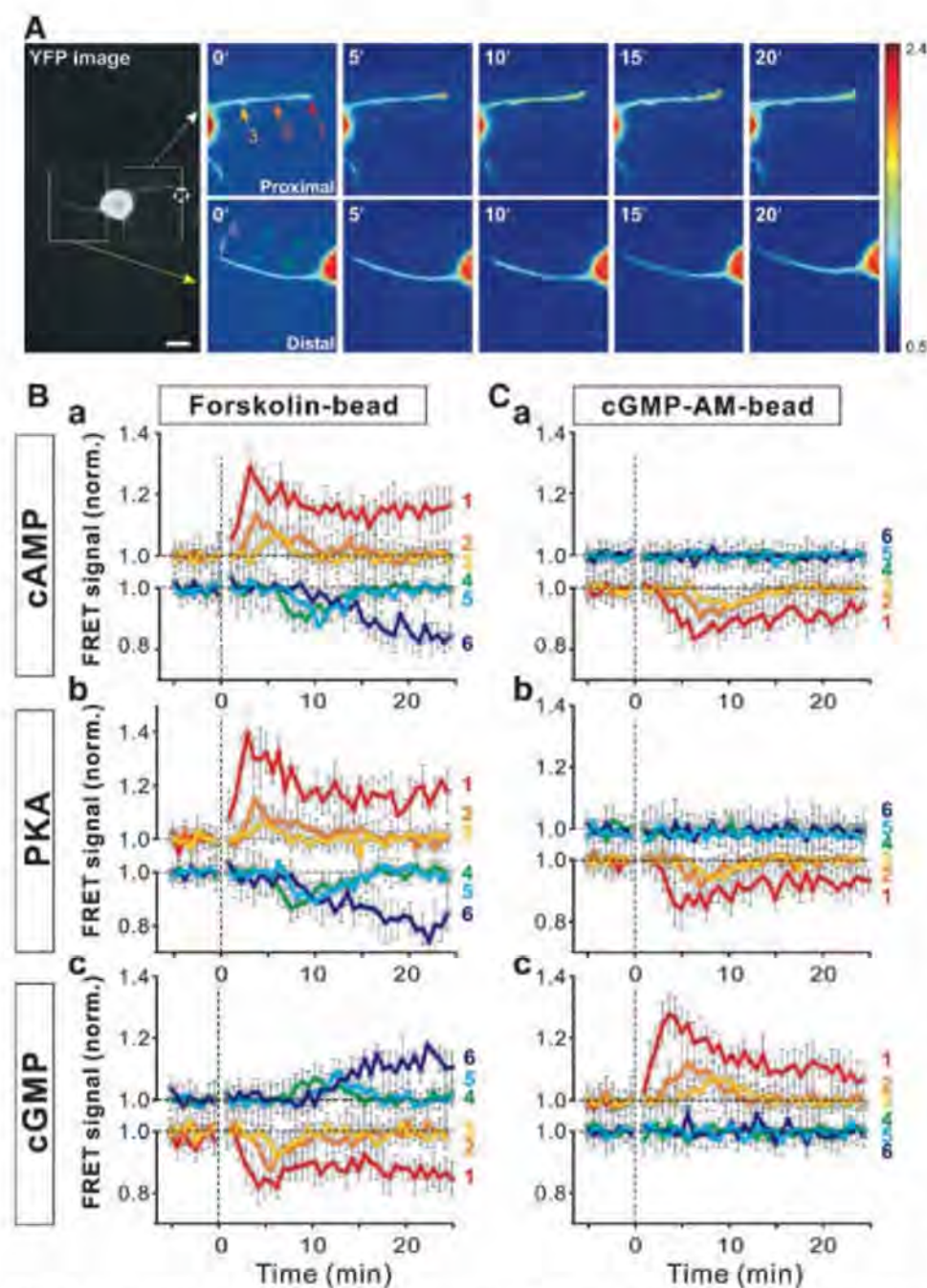
also regulate AC/sGC or selective PDEs (29, 33). This hypothesis is supported by the finding that KT5720 alone not only reduced the PKA activity, but also increased cGMP and reduced cAMP, whereas KT5823 alone elevated cAMP/PKA and reduced cGMP (Fig. 2G).

**cGMP antagonizes the phosphorylation of axon determinants.** To further explore the mechanisms underlying the antagonistic actions of cAMP and cGMP on axon initiation, we inquired whether this antagonism is reflected in the phosphorylation of LKB1 (12), GSK-3 $\beta$  (8, 16), and

Akt (8, 15), which are proteins that promote axon formation after their phosphorylation. Immunoblotting of lysates of cultured cortical neurons using phosphorylation site-specific antibodies showed that elevating cAMP synthesis with forskolin caused a PKA-dependent increase of LKB1 phosphorylation at serine 431 (S431) and of GSK-3 $\beta$  at S9 (Fig. 3C), and this effect correlated with the increased level of total LKB1 (12). Furthermore, when cultures that were plated on F-cAMP stripe were transfected with siRNA to LKB1 (12) and examined at 60 hours, the polarized population of these transfected neurons showed reduction in preferential axon initiation at the stripe boundary (fig. S2A). Forskolin caused no detectable Akt phosphorylation at S473 (Fig. 3C). Conversely, elevating cGMP by bath application of 8-pCPT-cGMP did not cause detectable reduction of LKB1 and GSK-3 $\beta$  phosphorylation (Fig. 3C), probably because of the low resolution of the immunoblotting method. However, in HEK-293T cells expressing an LKB1 construct, 8-pCPT-cGMP markedly reduced LKB1 phosphorylation and the total amount of LKB1 (fig. S2B). Activation of PKA activity by forskolin was confirmed in hippocampal cultures by a peptide-based assay (fig. S3, A and Ca), which also showed a dose-dependent reduction of the basal PKA activity in these cultured neurons by 8-pCPT-cGMP treatment (fig. S3B). The 8-pCPT-cGMP effects on the phosphorylation and stability of LKB1 depended on the PKA site 431, because they were absent in HEK-293T cells expressing LKB1 with a serine-to-alanine mutation (12) at this site (fig. S2B).

We further examined the mechanism underlying the effect of cGMP on PKA-dependent LKB1 phosphorylation. Bath-applied 8-pCPT-cGMP dose-dependently reduced the phosphorylation (at S431) and stabilization of LKB1 induced by forskolin in cultured cortical neurons (Fig. 3D). This antagonistic cGMP effect was abolished by either IBMX or rolipram (Fig. 3D), indicating that cGMP reduced the cAMP level primarily by PDE4 activation. The antagonistic effect of 8-pCPT-cGMP on LKB1 phosphorylation was largely absent when PDE-resistant Sp-8-Br-cAMPS was used instead of forskolin (Fig. 3E). The peptide-based PKA assay also confirmed these findings in cultured hippocampal neurons (fig. S3). Treatment with KT5823 resulted in a slight elevation of PKA activity in hippocampal neurons (fig. S3Cb) and LKB1 phosphorylation/stabilization in cortical cultures (Fig. 3F), consistent with the FRET imaging result showing that PKA/PKG inhibition could modulate the cAMP/cGMP level (Fig. 2).

**Perturbation of cAMP/cGMP affects neuronal polarization in vivo.** We also investigated the role of cAMP/cGMP in the development of cortical neurons in vivo by elevating cAMP and reducing cGMP in newly generated cortical neurons in rat embryos. This was achieved by in utero electroporation (34) of constructs expressing enhanced green fluorescent protein (EGFP) and specific siRNAs against either cAMP-selective phosphodiesterase 4D (PDE4D) (12) or soluble guanylate



**Fig. 4.** Local cAMP elevation induced long-range cAMP reduction, but local cGMP elevation had no long-range effect. (A) YFP fluorescence and FRET signals for cAMP in an ICUE-expressing hippocampal neuron at 16 hours, at different times (in minutes) after the contact by a forskolin-coated glass bead (dashed circle) in the bead-contacted (upper panels) and noncontacted (lower panels) neurite. Scale bar, 10  $\mu$ m. (B) Average FRET signals for cAMP, PKA, or cGMP in cells expressing ICUE (a), AKAR (b), or cGES-DES (c), respectively, detected at different locations along the forskolin bead-contacted neurite (1 to 3) and all other neurites (4 to 6). For bead-contacted neurites, the signals were measured at the bead contact site at the tip (1) and at sites 1/3 (2) and 2/3 (3) neurite lengths from the tip [see illustration in (A)]. For all other neurites, the signals were measured at sites 1/3 (4) or 2/3 (5) of neurite length from the soma, and at the tip of the neurite (6). Data represent average  $\pm$  SEM ( $n = 5$  to 10 cells, including all neurites). (C) Same as in (B), except that the bead was coated with cGMP-AM.



cyclase- $\beta 1$  subunit (sGC- $\beta 1$ ) (fig. S1B) into a subpopulation of neural progenitor cells at embryonic day 18 (E18). We found that cortical neurons expressing the siRNA against either PDE4D or sGC- $\beta 1$  exhibited impaired radial migration to the cortical plate (CP) in E21 embryos (fig. S4, A to C). Whereas most neurons had a single-neurite (unipolar) or two-neurite (bipolar) morphology in control E21 rat embryos, a large percentage of PDE4D or sGC- $\beta 1$  siRNA-expressing neurons were multipolar (fig. S4, D and E). The polarization defects correlated positively with the level of siRNA expression (fig. S4, F to H). Similar polarization and migration defects were also observed for PDE4D or sGC- $\beta 1$  siRNA-expressing neurons in postnatal day 0 (P0) rat cortices (fig. S5). Thus, elevating cAMP and reducing cGMP produced similar defects in developing cortical neurons in vivo. However, whether there is a causal relationship between the tightly linked processes of neuronal polarization and radial migration during this stage of development and whether cAMP/cGMP signaling is involved in both processes remain to be further elucidated.

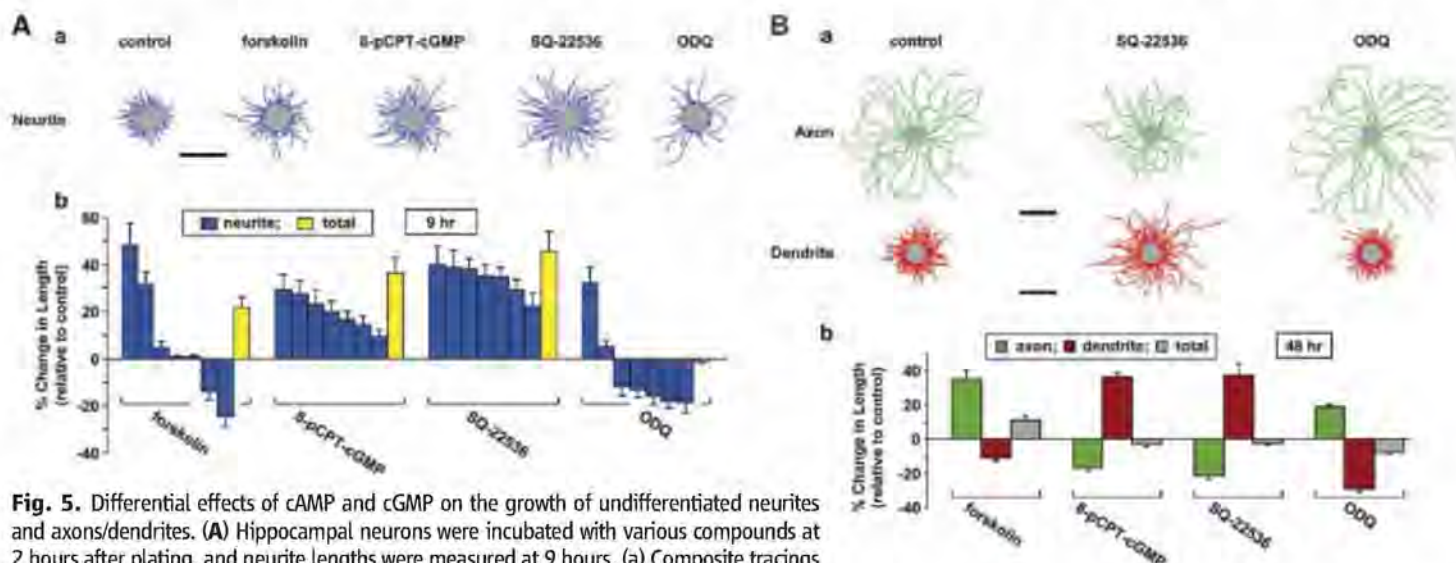
**Local cAMP elevation causes long-range cAMP suppression.** The reciprocal regulation between cAMP and cGMP could facilitate neurite differentiation along the axonal and dendritic route when cAMP and cGMP are locally elevated, respectively. To examine how this local cAMP/cGMP elevation affects the global cAMP/cGMP signaling of the entire neuron, we manipulated a glass bead coated with forskolin (SOM text) into contact with an undifferentiated neurite of hippocampal neurons expressing the FRET reporter for cAMP or PKA activity. We found a marked and persistent increase in cAMP or PKA signals at the bead contact site (Fig. 4, A, Ba, and Bb, and fig. S6) and transient cAMP/PKA signals along the

shaft of bead-contacted neurites, with decreasing amplitude and longer delay of onset at more proximal locations toward the soma (Fig. 4, A, Ba, and Bb, and fig. S6). The apparent spread of cAMP/PKA activity along the neurite was not caused by the diffusion of forskolin extracellularly, because no signal was observed when the forskolin-coated bead was placed in close proximity ( $<2 \mu\text{m}$ ) but not in direct contact with the neurite (fig. S6). After the forskolin-bead contact, we observed a striking reduction of cAMP/PKA signals in all other neurites (Fig. 4, A, Ba, and Bb, and fig. S6), with increasing extent of reduction and a longer delay of onset at more distal locations. In a reciprocal manner, in cells expressing the cGMP reporter cGES-DE5, the cGMP signal was reduced at the forskolin bead-contacted neurite but increased in all other neurites, with a pattern exactly mirroring that of cAMP/PKA (Fig. 4Bc).

When similar experiments were done using a glass bead coated with the membrane-permeable cGMP-AM (acetoxymethyl ester), we found a local elevation of the cGMP signal and a reduction of the cAMP/PKA signal at the bead-contacted neurite, but a complete absence of any long-range effect on either cGMP or cAMP/PKA in all other neurites (Fig. 4C and fig. S6). Because the cGMP-AM bead also induced a local cAMP/PKA reduction, the lack of effect in other neurites indicates that the long-range cAMP signaling is unidirectional, triggered only by cAMP elevation. The existence of long-range self-down-regulation of cAMP but not cGMP immediately suggests a mechanism for the formation of one axon and multiple dendrites: Axon induction by local cAMP elevation leads to suppression of axon formation and promotion of dendrite formation at all other neurites, whereas dendrite induction by local cGMP elevation has no long-range effect.

**Differential growth regulation by cAMP and cGMP.** Polarization of cultured hippocampal neurons begins with growth acceleration of a single undifferentiated neurite (1, 2, 4, 21), suggesting that axon initiation may be achieved by signals acting directly on specific cytoskeletal components (35–37) that accelerate neurite growth. Differential growth regulation may contribute to the antagonistic action by cAMP and cGMP on axon/dendrite differentiation. We thus examined the effect of global manipulation of cAMP/cGMP activities on the growth of undifferentiated neurites and axons/dendrites. Bath application of various pharmacological agents at 2 hours after cell plating and neurite length measurements at 9 hours showed that global cAMP elevation (with forskolin) or cGMP reduction (with ODQ) enhanced the growth primarily of one or two neurites (Fig. 5A), whereas global cGMP elevation (with 8-pCPT-cGMP) or cAMP reduction (with SQ-22536) resulted in relatively uniform growth promotion of all neurites (Fig. 5A). The asymmetry in neurite growth promotion by cAMP elevation may reflect the dominance of a single neurite that first acquired the highest cAMP elevation, together with long-range self-down-regulation of cAMP that suppressed the growth of most other neurites. By 48 hours after plating, global elevation of cAMP (or reduction of cGMP) increased the axon length but reduced the dendrite length, whereas global elevation of cGMP (or reduction of cAMP) had opposite effects (Fig. 5B), consistent with cAMP/cGMP antagonism. These results also suggest that axon and dendrite growth involve cAMP- and cGMP-dependent activation of specific cellular components, respectively.

**Discussion.** Many extracellular factors known to regulate neuron polarization, including brain-derived neurotrophic factor (BDNF) (8, 12), nerve growth factor (NGF) (9), Sema3A (38), netrin-I



**Fig. 5.** Differential effects of cAMP and cGMP on the growth of undifferentiated neurites and axons/dendrites. **(A)** Hippocampal neurons were incubated with various compounds at 2 hours after plating, and neurite lengths were measured at 9 hours. **(a)** Composite tracings of all neurites from 10 randomly sampled neurons in control and treated cultures. Scale bar, 50  $\mu\text{m}$ . **(b)** Percentage difference in neurite length (average  $\pm$  SD;  $n = 3$  to 5 cultures, 70 to 100 cells each) relative to the average neurite length for 100 neurons in parallel control cultures, for up to the first seven longest neurites (blue) and for all neurites (yellow). **(B)** Axon and dendrite lengths at 60 hours (compound added at 2 hours), determined by immunostaining with axon- and dendrite-specific markers, smi-312 and MAP2, respectively. **(a)** Composites of tracings of randomly sampled cells (axons, 25 cells, green; dendrites, 15 cells, red) in control and treated cultures. Scale bar, 100 (axons) and 50 (dendrites)  $\mu\text{m}$ . **(b)** Percentage difference of axon and dendrite lengths (average  $\pm$  SD;  $n = 3$  to 5 cultures, 70 to 100 cells each) relative to those in 100 control neurons in parallel cultures.



(10), Wnt (11), and laminin (6, 7), could modulate the cAMP or cGMP level in neurons (12, 38–43). Localized exposure to one or more of such factors may create a cytoplasmic asymmetry for axon/dendrite initiation. Perturbation of the cAMP/cGMP level resulted in neuronal polarization defects in the developing cortex (figs. S4 and S5), but the cAMP/cGMP-modulating extracellular factors that are responsible for polarizing the neurons in vivo remain to be identified. In a model for distinct actions of local versus global cAMP/cGMP signaling (fig. S7), we propose that axons and dendrites are induced via localized cAMP and cGMP signals, and local antagonistic interactions between cAMP and cGMP pathways ensure that axon initiation is accompanied by the inhibition of dendrite formation, and vice versa. The cAMP signal acts through phosphorylated LKB1 (12) and GSK-3 $\beta$  (8, 16) and their downstream effectors, which may converge with the PI3K pathway at several levels to promote axon initiation (15, 19, 44–46), whereas the cGMP signal suppresses axon formation via reciprocal down-regulation of cAMP/PKA-dependent phosphorylation of LKB1 and GSK-3 $\beta$  (Fig. 3), as well as specifically promotes dendrite growth (Fig. 5) via cellular processes yet to be identified (fig. S7).

Long-range inhibitory signaling in neurons has been reported. Local contact of a neurite with a target cell (47) or laminin-coated surface (6, 7), or local perfusion of a neurite with forskolin (48), all led to growth inhibition of distant neurites. This inhibitory effect may be caused by the long-range self-suppression of cAMP, although the mechanism underlying this long-range cAMP self-antagonism remains to be elucidated. The absence of long-range signaling resulting from local cGMP elevation suggests the framework of axon dominance signaling in the coordinated axon/dendrite

differentiation; whereas local cAMP/cGMP reciprocal regulation helps to channel the differentiation process along either an axonal or dendritic route, the long-range cAMP self-suppression ensures the formation of only one axon.

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#### Supporting Online Material

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## REPORTS

# Phase Transitions of Adsorbed Atoms on the Surface of a Carbon Nanotube

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Phase transitions of adsorbed atoms and molecules on two-dimensional substrates are well explored, but similar transitions in the one-dimensional limit have been more difficult to study experimentally. Suspended carbon nanotubes can act as nanoscale resonators with remarkable electromechanical properties and the ability to detect adsorption at the level of single atoms. We used single-walled carbon nanotube resonators to study the phase behavior of adsorbed argon and krypton atoms as well as their coupling to the substrate electrons. By monitoring the resonance frequency in the presence of gases, we observed the formation of monolayers on the cylindrical surface, phase transitions within them, and simultaneous modification of the electrical conductance.

Films of atoms or molecules adsorbed on surfaces exhibit many kinds of ordering that reflect interactions between the adsorbates as well as with the surface. One of

the simplest model systems, rare gases on bulk exfoliated graphite, exhibits a huge range of two-dimensional (2D) phenomena within the first adsorbed layer, including 2D melting, transitions

between solids that are either commensurate or incommensurate with the graphene lattice, and critical behavior (1, 2). Here, we explore the phase behavior of argon and krypton on carbon nanotubes, where the dimensionality of the substrate approaches the 1D limit, and report effects on the electronic conductance that reflect adsorbate-substrate interactions. We map out this phase behavior using nanomechanical resonators based on individual suspended single-walled nanotubes (SWNTs) (3–6). This platform combines remarkable electrical (7–9) and electromechanical (10–12) properties with extreme mass sensitivity (13–15) and allows simultaneous measurement of both the precise amount of adsorbed substance and its effect on the electrical

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properties. Our approach avoids problems of heterogeneity that have complicated previous explorations of this regime based on conventional techniques that require the use of bulk nanotube samples (16–18).

Suspended nanotube devices were made by prefabricating the electrodes and trench, and then growing the SWNTs in the last step (19) to avoid any chemical exposure that might contaminate the pristine nanotube surface (Fig. 1A). Mechanical resonances appear as sharp features in the current signal during frequency sweeps (20). The frequency  $f_{\text{res}}$  of each resonance decreases with increasing pressure  $P$  as gas adsorbs (20), as illustrated in Fig. 1B for Kr at 77 K. Equilibrium is established in seconds after a small pressure change (Fig. 1C), and  $f_{\text{res}}$  is a reproducible function of  $P$  (Fig. 1D).

We deduce the adsorbed mass by assuming that at a given temperature  $f_{\text{res}}$  varies as  $\rho^{-1/2}$ , where the total mass per unit length  $\rho$  is the sum of that of the bare nanotube,  $\rho_0$ , and of the adsorbates,  $\Delta\rho$ . (This assumption will be justified

below.) The fractional mass increase of the nanobalance is then  $\Delta\rho/\rho_0 = (f_0/f_{\text{res}})^2 - 1$ , where  $f_0 = \lim_{P \rightarrow 0} f_{\text{res}}$ . Because  $f_0$  is determined separately at each temperature  $T$ , any variation of the resonance frequency with  $T$ , such as might result from thermal expansion, is factored out. If  $m_C$  and  $m_{\text{ads}}$  are the atomic masses of carbon and the adsorbed species respectively, then the quantity

$$\varphi = (\Delta\rho/m_{\text{ads}})/(\rho_0/m_C) = m_C/m_{\text{ads}} [(f_0/f_{\text{res}})^2 - 1] \quad (1)$$

is the number of adsorbed atoms per carbon atom; Fig. 1E shows an example of an isotherm of  $\varphi$  versus  $P$  derived in this way.

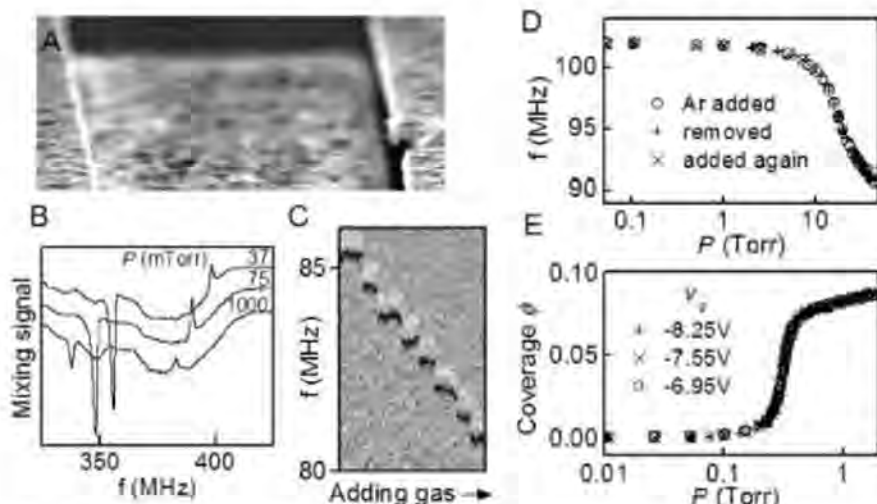
The assumed  $\rho^{-1/2}$  scaling of  $f_{\text{res}}$  requires that the change in elastic properties is negligible compared with the fractional change in mass. This assumption can be made because the covalent C–C bond is two orders of magnitude stronger than the van der Waals attraction between adsorbates. We also must assume that the mass is distributed uniformly over the nano-

tube surface, which would not be the case if part of the surface were contaminated, or if a denser phase appeared preferentially at the ends or in the middle in response to long-range forces. Nonuniformity would cause different vibrational modes to shift in different ways. However, we have found that  $f_0/f_{\text{res}}$  does not depend on which mode is used and also that it is insensitive to substrate gate voltage  $V_g$ , up to about 8 V (see, for example, Fig. 1E). Taken together with the results described below, these observations indicate that many of our devices consist of single-walled nanotubes in which  $\varphi$  is a good measure of the coverage (number of adsorbates per surface atom) and that the coverage is uniform.

Isotherms of  $\varphi$  versus  $P$  for Ar are dominated by a large, smooth step, as shown in Fig. 2 for device YB3. A similar step is well known in conventional volumetric isotherms on bulk exfoliated graphite and is characteristic of the densification of a supercritical 2D fluid (F), which occurs above 56 K, the 2D liquid (L)–vapor (V) critical point of Ar on graphite (2, 21, 22). The colder Ar isotherms also show a second, smaller step at  $\varphi \approx 0.24$  (see the inset of Fig. 2). A similar second step is seen for Ar on graphite (22) when the fluid freezes to a 2D incommensurate solid (IS). Accordingly, we anticipate an L+V coexistence region at lower temperatures as indicated by the dotted line, and we expect the F+IS coexistence region to have the form indicated by the dashed lines. We note that corresponding features occur at higher pressures on nanotubes than on graphite, reflecting the expected weaker binding of atoms to a nanotube surface than to bulk graphite (23).

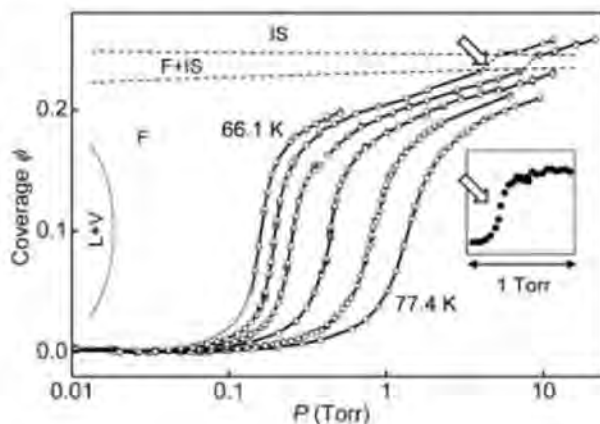
We now discuss how this phase behavior changes for Kr, which is larger and more polarizable than Ar. Isotherms of Kr on device YB3 exhibit a dramatic vertical step followed by two smaller steps (Fig. 3). Again, these resemble conventional volumetric isotherms of the same substance on exfoliated graphite (24) but shifted to higher pressures. The size and sharpness of the large step implies not only a first-order phase transition but also excellent substrate homogeneity, consistent with the absence of grain boundaries or imperfections on the surface of this SWNT. The first plateau, between the first two steps, is narrow and easily missed; it is resolved in the 77.4 K data here but not the 73.7 K data.

Whereas Ar does not form any commensurate phase on graphite, Kr condenses from a low-density 2D vapor (V) to a commensurate solid (CS) with one Kr atom per six C atoms (25), as indicated in the left inset to Fig. 3. At higher pressure, it converts to an IS (26). The first plateau in our Kr isotherms, whenever resolved, is centered at  $\varphi = 1/6$ , corresponding to the coverage of the commensurate solid and implying that the first step is a V–CS transition. The highest plateau reached is likely to be the IS, whereas the intermediate plateau is of unknown nature but may be related to a proposed reentrant fluid phase (27).



**Fig. 1.** Detecting adsorption on a single vibrating nanotube. (A) Electron micrograph of a nanotube spanning a 2- $\mu\text{m}$  wide trench between Pt contacts. (B) Line traces of the mixing current signal showing resonances shifting with Kr gas pressure for device YB3 (0.5- $\mu\text{m}$  gap) at 77.4 K and  $V_g = 9.58$  V. (C) Grayscale of the mixing current signal for a series of frequency sweeps taken during a sequence of Kr pressure increments, one roughly every 3 min, demonstrating rapid equilibration, for device YB1 (2- $\mu\text{m}$  gap) at 77.4 K. (D) Variation of resonance frequency with Ar pressure for YB1, showing high reproducibility. (E) Variation of coverage parameter  $\varphi$  obtained using Eq. 1 with Kr pressure for YB1, demonstrating little sensitivity to gate voltage.

**Fig. 2.** Isotherms of coverage parameter  $\varphi$  for Ar on device YB3. The temperatures are 66.1, 67.7, 68.8, 71.0, 73.9, and 77.4 K. The large step occurs within the supercritical fluid (F), with a first-order L–V transition expected below a critical point at  $\sim 0.01$  Torr. The smaller step, a separate measurement of which is shown in the inset, occurs at the transition to an IS. Dotted and dashed lines indicate boundaries of coexistence regions.





The fact that  $\phi$  takes the value of  $1/6$  for the commensurate phase strongly indicates that the assumption behind Eq. 1 that  $f_{\text{res}} \propto \rho^{-1/2}$  is justified for this device. Some other devices, including YB1 (Fig. 1E), showed similar responses but exhibited relatively small values of  $\phi$ . This difference can be explained if these devices have a reduced ratio of available surface area to nanotube mass, which would be the case for a multi-walled nanotube or if part of the nanotube's surface were contaminated.

The identification of commensurate and incommensurate 2D solids on the cylindrical nanotube surface raises many interesting questions. The solid is subject both to the curvature, which breaks the isotropy of the graphene lattice, and to the cylindrical boundary condition. It may be rolled seamlessly like the underlying graphene, or alternatively it may contain a domain wall running along the nanotube. For the CS, which is in registry with the carbon surface, the seamless case occurs only when  $(N - M)/3$  is an integer, where  $(N, M)$  is the nanotube's roll-up vector. Interestingly, this is precisely the same condition as for the nanotube to be metallic (28). From the

$V_g$  dependence of its conductance  $G$  (right inset to Fig. 3), we identify YB3 as a small-gap metallic nanotube that obeys this condition.

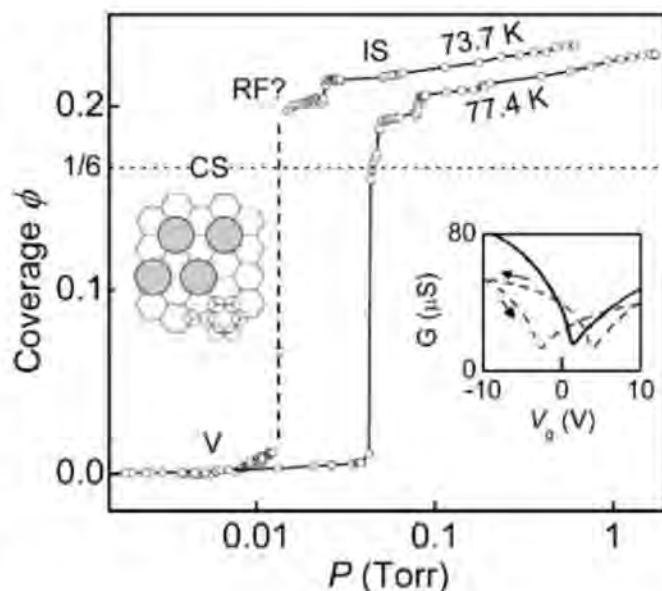
Because we can measure the electrical properties of the SWNT simultaneously with the mass absorption, we can quantitatively investigate the coupling between adsorbates and electrons. This is not possible in conventional adsorption experiments. Figure 4 shows the  $\phi$ - $P$  isotherm of Kr at 77 K for another device, YB8. It exhibits a large, sharp step at about 16 mTorr similar to that into YB3. (The smaller step pressure in YB8 can be explained by a larger binding energy due to a larger nanotube diameter.) The upper left inset shows  $G$ - $V_g$  characteristics measured at pressures on either side of the phase transition. The conductance is suppressed at the higher pressure for positive  $V_g$ . The other trace in the main panel shows the resistance measured at a fixed positive gate voltage. It increases gradually at low coverages and jumps suddenly at the transition. This behavior is reproducible and reversible.

One immediate consequence of this observed coupling is that we can investigate the dynamics of such a phase transition. The right inset to Fig. 4

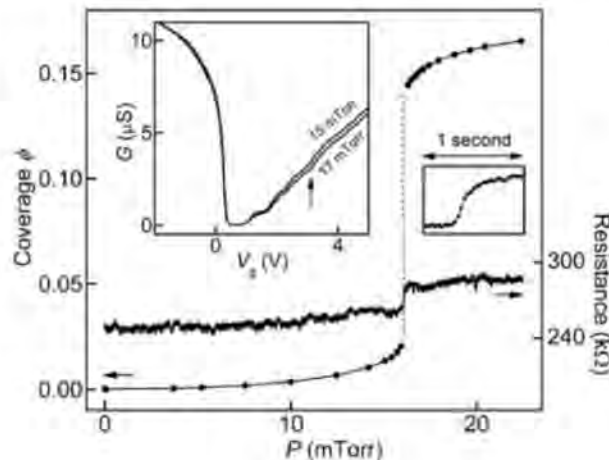
shows the resistance monitored with a 10-ms instrumental response time as the Kr pressure is increased rapidly across the transition. The step occurs in about 0.1 s, indicating that this is the intrinsic time scale of the phase transition on the SWNT.

Analysis of the  $G$ - $V_g$  characteristics indicates that this SWNT has a small gap ( $\sim 60$  meV) and that the conductance is limited by tunneling across the gap at positive  $V_g$  but not at negative  $V_g$  (20). The larger decrease in conductance at the phase transition for positive  $V_g$  could thus be related to an increase in the gap. We note that the CS has a reciprocal lattice vector that connects the Dirac points in the graphene Brillouin zone, and hence coherent scattering from commensurate Kr offers a possible mechanism for modifying the gap. Also, the Kr atoms will be statically polarized by the gate-induced electric field perpendicular to the nanotube surface (20), thereby modifying their interactions with each other and with the substrate at high gate voltages. Further experiments of this type could yield many more important insights into the interaction of adsorbed substances with the electrons in graphitic carbon.

**Fig. 3.** Adsorption of Kr on device YB3, showing first-order phase transitions. (The 73.7 K isotherm has incomplete data at the dashed line.) The dotted horizontal line at  $\phi = 1/6$  corresponds to the expected coverage of 1 adsorbate per 6 carbon atoms in a commensurate solid on a clean SWNT. The left inset shows the commensurate arrangement of adsorbates (shaded circles) sitting on every third carbon hexagon. The right inset shows conductance versus gate voltage (solid: vacuum; dashed: in air, where there is hysteresis indicated by the arrows) at room temperature, whose form is that of a small-bandgap nanotube.



**Fig. 4.** Combined mass and transport measurements on device YB8 (1-μm gap) exposed to Kr at 77 K. Measurements are shown of both coverage parameter  $\phi$  (left axis) and resistance  $R$  (right axis) as a function of pressure. The left inset shows conductance versus gate voltage at pressures just below (15 mTorr) and above (17 mTorr) the transition, with an arrow indicating the gate voltage of +3.1 V at which  $R$  was measured. The right inset shows resistance versus time during a rapid upward pressure sweep across the transition.



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#### Supporting Online Material

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Figs. S1 to S3  
References

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# Spontaneous and X-ray–Triggered Crystallization at Long Range in Self-Assembling Filament Networks

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We report here crystallization at long range in networks of like-charge supramolecular peptide filaments mediated by repulsive forces. The crystallization is spontaneous beyond a given concentration of the molecules that form the filaments but can be triggered by x-rays at lower concentrations. The crystalline domains formed by x-ray irradiation, with interfilament separations of up to 320 angstroms, can be stable for hours after the beam is turned off, and ions that screen charges on the filaments suppress ordering. We hypothesize that the stability of crystalline domains emerges from a balance of repulsive tensions linked to native or x-ray–induced charges and the mechanical compressive entrapment of filaments within a network. Similar phenomena may occur naturally in the cytoskeleton of cells and, if induced externally in biological or artificial systems, lead to possible biomedical and lithographic functions.

One-dimensional (1D) objects in solution, such as carbon nanotubes (1), filamentous viruses (2), and rigid molecules (3), can spontaneously form orientationally ordered domains or networks as a result of their shape. This excluded volume effect is useful in the design of devices, liquid crystals, high-strength materials, bioactive hydrogels, and other functional structures. In biological systems, the bundling, orientation, and mechanical networking of 1D cytoskeleton components such as filamentous actin and microtubules mediate cellular events such as mitosis, protein transport, and signal transduction (4–9). Small-angle x-ray scattering (SAXS) can provide information on the size, shape, and symmetry of the internal domains of materials (10, 11) and is extremely useful to study structures with the length scales of 3D networks formed by nanoscale filaments (4). However, x-ray beams can cause irreversible chemical changes that lead to detectable products at high fluxes and affect the formation of 3D structures (12–14). The typical

mechanisms involve ejection of energetic electrons from molecules via the well-known photoelectric, Compton, and Auger effects and form free radicals and charged molecules, as well as chemical reactions (15–19).

We report the spontaneous and x-ray–triggered crystallization of supramolecular filaments within 3D networks at unexpectedly large distances (up to 320 Å). The filaments described here are formed by self-assembly in water of a synthetic molecule containing the short peptide sequence Ala<sub>6</sub>Glu<sub>3</sub> (A<sub>6</sub>E<sub>3</sub>) covalently grafted to an alkyl chain of 16 carbons (fig. S1) (20). A representative cryogenic transmission electron microscopy (cryo-TEM) image is shown in Fig. 1A, revealing cylindrical nanofibers measuring ~102 Å in diameter corresponding to twice the fully extended length of the molecules. The length of the filaments cannot be directly obtained from cryo-TEM, but it is estimated to be on the scale of tens of micrometers. The combined effect of intermolecular hydrogen bonding among the peptide segments and hydrophobic collapse of hydrocarbon tails in these molecules leads to formation of the 1D nanostructures in dilute solution (21, 22).

We observed that the SAXS profiles of 0.5 weight percent (wt %) solutions dramatically changed upon continuous x-ray exposure. Figure 1B displays 50 sequential SAXS profiles of the same spot with an exposure time of 4 s each, plotted on a double-logarithmic scale. The first spectrum (red) shows a typical scattering profile of cylindrical objects in solution, as suggested by the –1 slope in the low *q* region and a diffuse form

factor peak around 0.1 Å<sup>–1</sup> (fig. S2) (20, 23). Successive irradiation on the same spot yielded a series of Bragg peaks. The relative positions of the peaks follow the *q*/*q*<sup>\*</sup> ratios of 1 : √3 : √4 : √7 : √9 : √12 (where *q*<sup>\*</sup> is the principal peak position), characteristic of a highly ordered 2D hexagonal lattice (space group *p6mm*). The development of scattering profiles is characterized by the emergence and continued increase in intensity of the principal Bragg peak, as well as the appearance of additional peaks at higher *q* values. Figure 1C shows the 2D scattering patterns of the first and the last exposures, revealing the disorder-to-order transition. The low volume fraction of filaments (0.5 wt %) indicates that these hexagonally packed 1D objects must exist as bundles. We measured the final values of full width at half maximum and used the Scherrer equation to estimate a bundle size. The calculation yields a value of about 1 μm; however, given the size regime the absolute value obtained from the Scherrer equation is of questionable accuracy. The Debye-Scherrer ring-like pattern after x-ray exposure is typical of scattering from a powder sample, implying that these hexagonal crystalline bundles are randomly distributed in solution (Fig. 1D).

We found that this x-ray–triggered structural rearrangement only occurred at relatively low concentrations. Figure 2A shows a similar disorder-to-order transition observed at 1 wt % upon continued x-ray irradiation. However, at higher concentrations (2 wt % and higher), the Bragg peaks could be observed without the need for continuous x-ray irradiation (Fig. 2, B and C). The hexagonally stacked filaments at 2 wt % and 5 wt % were stable during x-ray exposure, and their corresponding Bragg peak positions did not shift by more than 3% with accumulated exposure time.

These observations suggest that hexagonal stacking of filaments at higher concentration is a spontaneous process and not associated with x-ray irradiation. We plot the x-ray profiles corresponding to the last exposure for various concentrations in Fig. 2D. Five to seven Bragg peaks are registered in each scattering profile and have the expected relative ratios of hexagonal structures. The Bragg peak positions shift smoothly to higher values of *q* with an increase in peptide concentration in both spontaneous and x-ray–triggered hexagonal structures. These changes correspond to a decrease in interfilament separation as the concentration rises (Fig. 2E) and suggests the spontaneous and x-ray–triggered hexagonal structures emerge through similar mechanisms.

To determine whether x-ray beam heating could contribute to this structural disorder-to-order

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transition, we studied the 1 wt %  $\text{AgE}_3$  solution at 25° and 40°C. We found that Bragg peaks did not appear after the first 4-s exposure at 40°C and that the overall scattering profiles underwent a similar transition as those obtained at 25°C (fig. S3) (20). Figure 2F shows the relative change in the  $q^*$  peak intensity plotted with respect to the exposure time; we observed no obvious difference between 25° and 40°C. In addition, Caffrey and co-workers reported only a 0.16°C temperature rise as a result of continuous x-ray exposure of Milli-Q (Millipore) water (16). Beam heating results typically in only a small temperature change because of the rapid dissipation of heat into the local environment; therefore, one does not expect this thermal effect alone to contribute to the observed structural transition (fig. S3).

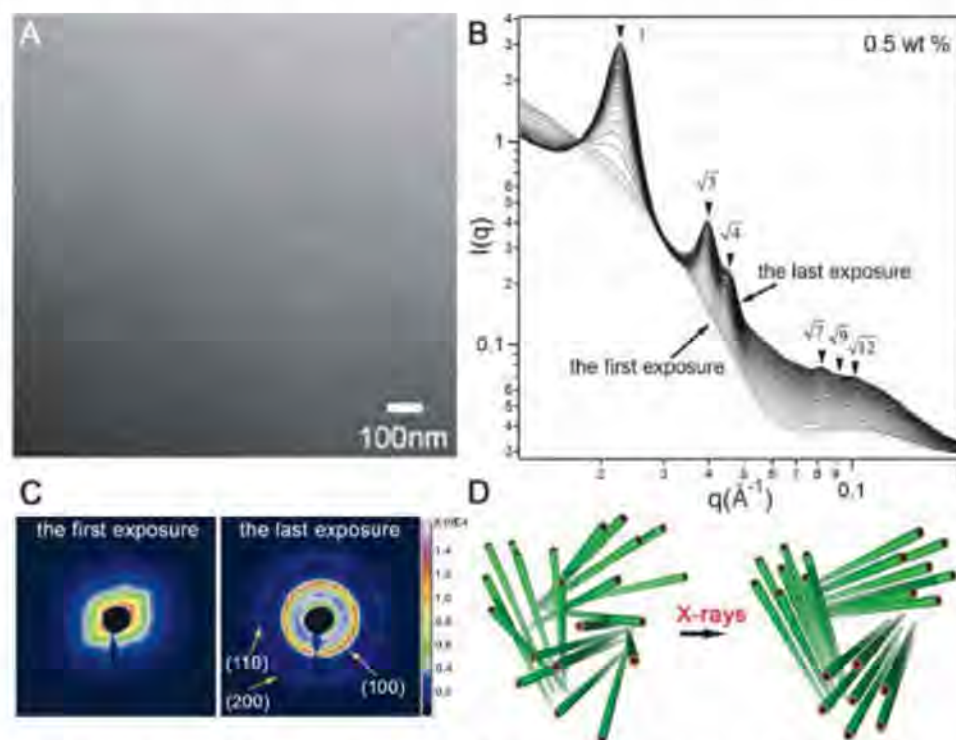
Another important observation was the reversal of the x-ray-triggered crystalline structures to a disordered state after removal of the beam. Macroscopically, the 1 wt % solution in the capillary was clear before x-ray irradiation. After x-ray exposure (accumulated 200 s), the spot of interest became opaque (marked with a white arrow in Fig. 3A). This appearance of turbidity is indicative of a transition between two states with different structural order, possibly because of scattering by crystalline filament domains. After removal of the x-ray beam, the solution gradually became clear and completely lost its opacity within about 40 min (Fig. 3, A to D, and fig. S4). This reversible opacity was further investigated with scattering experiments. Figure 3E shows the transition from disordered filaments (black curve, the first 4-s exposure) to the hexagonally ordered bundles (red curve, the last 4-s exposure). Two hours after removing the x-ray beam, we collected scattering profiles on the same location. We found that all peaks corresponding to hexagonal filament stacking vanished (green curve), thus suggesting the loss of positional order. Restarting x-ray irradiation causes the reappearance of Bragg scattering peaks (blue curve). This reversibility is also observed when adjacent regions to the originally irradiated spot are exposed to x-rays. This experiment suggests that reversibility is not caused by simple diffusion of chemically unmodified material into the originally irradiated spot. In fact, analytical high-performance liquid chromatography (HPLC) and mass spectrometry of samples after a large x-ray dose confirmed that chemical damage is not involved in the observed reversible phenomenon (figs. S10 to S36) (20).

In order to gain more insight into the observed x-ray-induced phenomenon, we studied the effects of beam flux and photon energy. A longer exposure time was required to induce this disorder-order transition when the x-ray beam was gradually attenuated (Fig. 3F). However, in order to induce filament ordering, a larger accumulated dose [supporting online material (SOM) text S2 and S6 and figs. S5 and S6] (20) was needed when samples were exposed to x-rays at a lower dose rate (a more attenuated beam) (Fig. 3G). Eventually, at a beam attenuation of 0.012,

the Bragg peaks corresponding to the hexagonally ordered filaments did not appear even with an accumulated 1120-s exposure and a total dose about twice as large as that needed to induce the filament ordering with a nonattenuated beam [ $4.39 \times 10^4$  grays (Gy)] (fig. S6) (20). Radiation damage by x-rays has been reported as more severe when samples are irradiated with a fixed accumulated dose but at a lower dose rate [the inverse dose-rate effect (18)]. Thus, in this context, if chemical damage did occur and the resulting products were responsible for filament ordering, the same accumulated dose at a lower dose rate should have enhanced the effect. These dose rate-dependent experiments again support that a reversible process is involved. Filament ordering can be induced by using x-ray photons with a lower energy (9 keV versus 15 keV) (fig. S7) (20), which suggests that much weaker beams can be used to promote ordering given that lower energy photons have a higher mass-energy absorption coefficient.

An unexpected feature of the observed hexagonal crystalline structures formed spontaneously or triggered by x-rays is the large  $d$  spacing among filaments (Fig. 2E). The center-to-center spacing is as large as 320 Å for 0.5 wt % solutions (x-ray-triggered) (Fig. 2F). Given the 102-Å diameter of

the filaments, the wall-to-wall distances measured range between 218 Å and 90 Å for concentrations of 0.5 wt % and 5 wt %, respectively. These distances are much larger than those reported for cytoskeleton filaments and DNA strands (4, 24, 25). In these systems, the distances measured are in the range of 30 to 55 Å and always in the presence of multivalent counterions that should introduce attractive forces among like-charge objects. The multivalency of added counterions, not added in our systems, is critical to induce positional ordering through attractive forces in these biomolecular systems. In one example, Safinya and co-workers have observed both hexagonal and necklace bundles in microtubule filaments after adding multivalent counterions (4). The structures are viewed as resulting from the balance among electrostatic repulsion and attraction as well as hydration forces (repulsive) and van der Waals interactions. Because multivalent counterions are not added in our systems, attractive electrostatic interactions cannot be considered as offsetting repulsive forces among the filaments. At the same time, van der Waals forces are not expected to stabilize repulsions at the interfilament distances of hundreds of angstroms observed here. We therefore hypothesize that crystalline stacking of filaments emerges from the minimization of



**Fig. 1.** X-ray irradiation triggered crystallization of self-assembling filaments at 0.5 wt % aqueous solutions of a peptide amphiphile. (A) Representative cryo-TEM image of filaments formed in 0.5 wt % solutions of peptide amphiphile. (B) A series of 50 consecutive scattering profiles of 0.5 wt % solutions obtained from the same sample area with 4-s exposure each (dose rate: 4806 Gy s<sup>-1</sup>). The measurements were performed at room temperature with a monochromatic x-ray at 15 keV, and the typical incident x-ray flux on the sample was  $\sim 1 \times 10^{12}$  photons per s with a 0.2 mm-by-0.3 mm collimator (samples were placed inside quartz capillaries of a diameter of 2 mm). The first and the last spectra are colored red and green, respectively. (C) The 2D scattering patterns corresponding to the first and last exposures in (B). (D) Schematic representation of x-ray-triggered organization of peptide filaments from a disordered state to a hexagonally ordered state.



repulsive electrostatic interactions balanced by other attractive forces associated with network structures.

To understand the observed disorder-to-order transition, we considered first the origin of bundle formation. Our insight is that bundle formation from filaments with very high aspect ratios must occur during their supramolecular growth, because these extremely long and networked filaments are unlikely to reorganize into bundles on short time scales once they are formed. TEM reveals the coexistence of very short and very long filaments at the very early stages of self-assembly (Fig. 4H). Based on this observation, we hypothesize that long filaments formed at early stages create a stable network that templates the formation of bundles as growth of short filaments continues. As depicted in Fig. 4, A to D, this “templating” effect can be envisioned as further parallel growth of filaments around the originally formed network. At the moment the precursor network is formed, topological constraints arising from interlocking of filaments would limit their mobility within a tubelike channel around each of them (Fig. 4A). Thus, the dimension of the channel would actually limit the size of the bundles formed. Long filaments can only move freely within their own channels, whereas shorter filaments can move in and out without any restrictions. Newly formed long filaments will be preferentially confined within one of these channels because of their local rigidity and high aspect

ratio, as well as the long-range electrostatic repulsions among the filaments (Fig. 4B). We assume that the growth of spatially correlated filaments in the limited space provides the foundation for the observed filament stacking. At 1 wt % or lower, subsequent filament growth within the space-confined channels eventually results in formation of loosely packed filament bundles that lack positional order (Fig. 4C). The increasing number of filaments within the bundles as concentration is increased can be readily observed by cryo-TEM (Fig. 4, E to G). We suggest that, beyond a critical concentration, electrostatic repulsions within bundles combined with our hypothesized network forces lead to hexagonal ordering of filaments (Fig. 4D). The interactions between these like-charge filaments depend not only on the interfilament distance and their mutual orientation but also on the 3D structure of the electric double layers arising from condensed and mobile ions. The theoretical analysis of electrostatic interactions within the bundles would require solving Poisson’s equation with boundary conditions for a system of parallel filaments,

$$\nabla \cdot (\bar{\epsilon} \bar{E}(\vec{r}) + \bar{P}(\vec{r})) = \rho(\vec{r})$$

where  $\bar{\epsilon}$  is the dielectric permittivity tensor,  $\bar{E}(\vec{r})$  is the electric field that determines the strength of electrostatic interactions,  $\bar{P}(\vec{r})$  describes the polarization of dielectric interfaces, and  $\rho(\vec{r})$  is the free charge density. For systems with high sur-

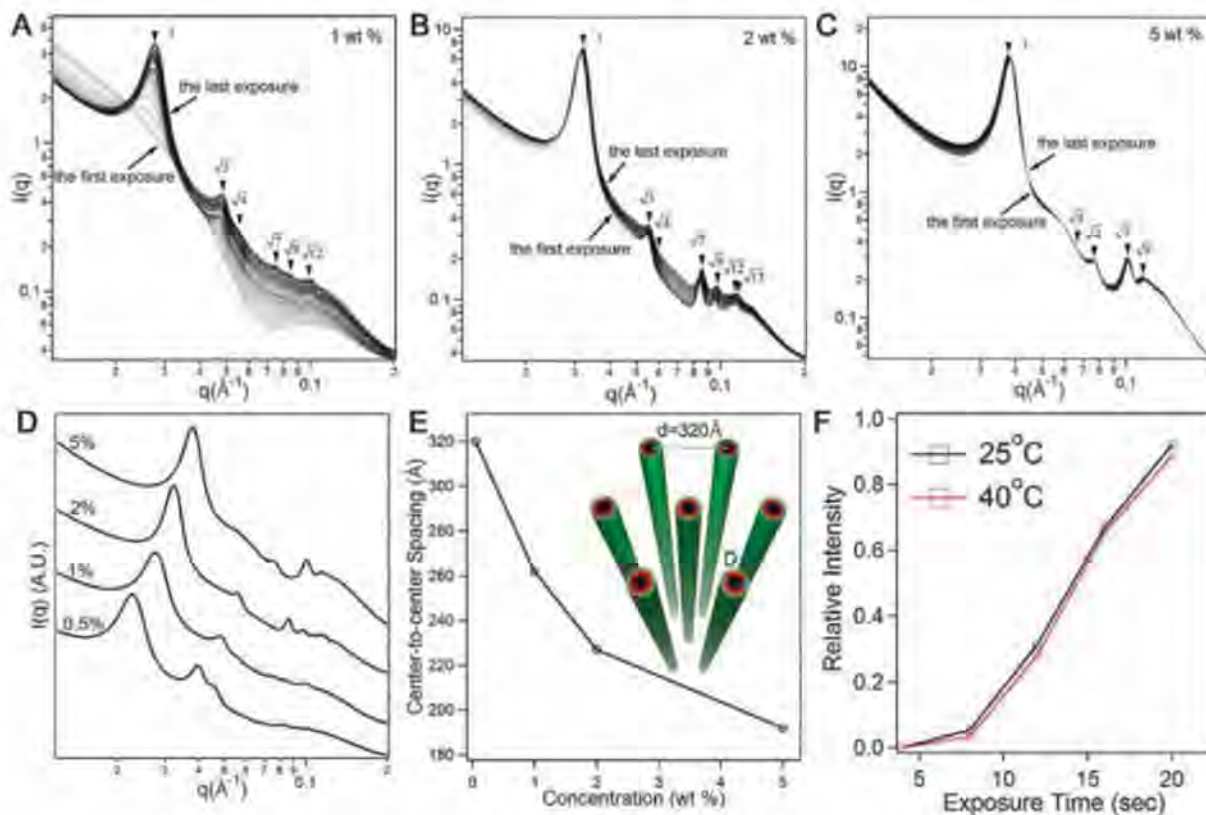
face charge in the presence of dielectric interfaces, charge correlations and interface polarization lead to intractable mathematical problems (26), clearly beyond the scope of this work.

The crystallization at long range driven by 200-s exposure to x-rays supports strongly the presence of bundles even in very dilute networks. The gradual rise in intensity of the principal Bragg peak suggests that more crystalline bundles are being formed upon continuous x-ray exposure. Also, on the basis of the Scherrer equation, the observed decrease with further exposure in full width at half maximum of this peak suggests that crystalline bundles are increasing in size. During this period of time, we do not expect reorganization of the extremely long filaments across a large volume, and therefore crystallization must be occurring locally within the bundles. At the same time, the observed reversibility between disordered and hexagonally ordered states at low concentrations suggests that interfilament forces must be changing as a result of x-ray exposure. X-ray irradiation is likely to increase the charge density on filament surfaces (18, 19, 27), thus altering the balance of forces within the bundles of the dilute network.

This new balance of forces induced by x-rays must resemble that prevailing in the spontaneous crystallization of bundles at high concentration. If electrostatic repulsive forces among the filaments are mediating crystallization at long range, they must be acting in concert with a balancing at-

**Fig. 2.** Concentration dependence and temperature effect of x-ray-triggered filament crystallization.

(A) Set of 50 consecutive scattering profiles from 1 wt % aqueous solutions (exposure time: 4 s). (B) Set of 30 consecutive scattering profiles from 2 wt % solutions (exposure time: 2 s), and 5 wt % solutions (exposure time: 1 s). (C) [The dose rate for the experiments in (A) to (C) was 4806 Gy s<sup>-1</sup>.] The first and the last profiles in (A) to (C) are colored red and green, respectively. (D) A replot of x-ray scans corresponding to the last exposure at concentrations of 0.5 wt %, 1 wt %, 2 wt %, and 5 wt %. (E) A plot of center-to-center spacings among filaments as a function of peptide amphiphile concentration (the inset shows a schematic representation of the largest spacing observed among filaments in 0.5 wt % solutions). (F) Effect of temperature on x-ray-triggered ordering of the filaments in a 1 wt % solution (the relative intensity is given by  $(I_n - I_0)/I_0$ , where  $n$  denotes the



accumulated exposure time and  $I_0$  is the intensity value at the location of the principal Bragg peak in the first scattering profile obtained after x-ray irradiation).



tractive force. We hypothesize that this attractive force could be rooted in the structural integrity of the entire network. The spatial confinement imposed by neighboring bundles in the network acts effectively as an "attractive" force balancing repulsion among the like-charge filaments. The stability of these networks containing crystalline bundles of like-charge filaments may originate in tension linked to repulsive forces within each bundle balanced by compression forces associated with the network. In this context, it is interesting that crystallization within the bundles provides a mechanism to minimize the local tension from repulsive forces. This balance is reminiscent of that existing in self-stabilizing tensegrity networks (8, 9).

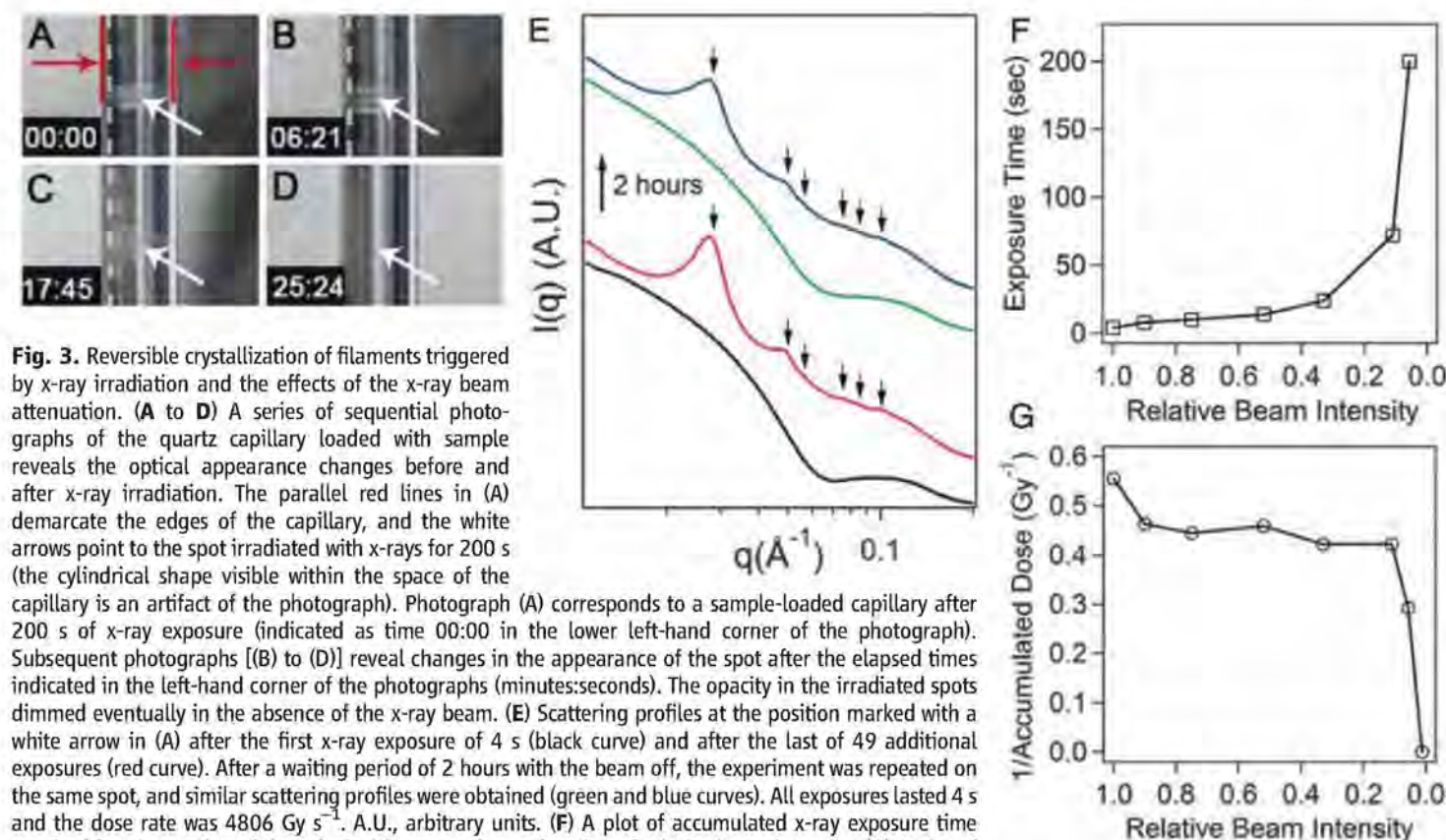
We carried out experiments to demonstrate the involvement of repulsive electrostatic forces in crystallization at long range within the filament bundles. These experiments involved adding NaCl and  $\text{CaCl}_2$  to 1 wt % solutions in order to screen electrostatic forces among like-charge filaments. As the amount of NaCl was increased, the primary Bragg peak developed more slowly during continuous x-ray irradiation (Fig. 4I and fig. S8) (20). At a concentration of 50 mM NaCl or higher, the x-ray-induced crystallization was not observed at all even after an accumulated exposure time of 120 s. Figure 4J shows 50 sequential scattering profiles of 1 wt % solution in the presence of 50 mM NaCl. When the divalent salt  $\text{CaCl}_2$  was used even in concen-

trations as low as 5 mM, the crystalline structures were never observed. Addition of 5 mM NaOH can promote filament ordering at 1 wt % without exposure of samples to x-ray irradiation (fig. S9) (20). NaOH can increase charge density on the acidic nanofiber surfaces by deprotonating COOH groups in glutamic residues. These observations support our hypothesis that electrostatic repulsions mediate crystallization of the filaments at long range. Our experiments also suggest that the observed reversible x-ray triggered crystallization results from increasing charge density on filament surfaces.

To investigate further whether this charge density increase is associated with permanent chemical modifications by the x-ray photons, we carried out chemical analysis on a sample irradiated with a large dose of x-rays. Even when about 60% of the total volume of a sample was irradiated with a dose 44 times greater than is needed to generate filament ordering, chemical changes were still not observed by using an ultraviolet-visible light detector. Only mass spectrometry detection could reveal a very small amount of chemically modified by-products (SOM text S10 and figs. S10 to S36) (20). In acidic, air-saturated aqueous solutions, the dominant species of water radiolysis are hydroxyl radicals ( $\cdot\text{OH}$ ) and hydrated electrons ( $e_{\text{aq}}^-$ ); both could potentially react with peptide backbones and side chains in various ways and yield products such as ammonia, keto acids, aldehydes, and other peptide derivatives

(19). Among these products,  $\alpha$ -hydroxyl carboxylic acids and  $\alpha$ -keto acids could potentially contribute to an increase in charge density because of their slightly lower  $\text{p}K_a$  (where  $K_a$  is the acid dissociation constant) values. Interestingly, detailed chemical analysis using mass spectrometry indicates that hydroxylation products are only associated with the alkyl tails and the first alanine residue adjacent to the tail. Our data also show that the very small amount of  $\alpha$ -hydroxylation occurring in the glutamic acid residues results in main-chain cleavage and formation of amide-terminated products (SOM text S10 and figs. S10 to S36) (20). We conclude that none of these species contributes substantially, if at all, to the increase in charge density on the surfaces of filaments.

Because oxygen plays a very important role in mediating this irreversible chemical reaction to generate the  $\alpha$ -hydroxylation products, we performed experiments in an oxygen-free solution and found that x-ray irradiation still induced ordering of filaments (fig. S37) (20). This experiment provides additional evidence that permanent chemical damage mediated by soluble oxygen in water is not necessary to facilitate filament ordering. In addition, we have observed filament ordering in other peptide amphiphile molecules with different sequences, for example, VVAEEGGREDKETV, VVAEEGGTKREEVD, and AAEEGGREDKETV (figs. S38 to S40) (20, 28), further suggesting that the mechanism of charge density increase





on filament surfaces is not specifically linked to glutamic acid residues but is instead a more general phenomenon.

On the basis of evidence described above, we propose that the main mechanism responsible for an increase in charge density on peptide nanofiber surfaces is linked to the reversible ionization of the COOH groups by x-ray irradiation. Because we obtained direct experimental evidence for filament ordering as a result of carboxyl ionization with strong base, we speculate that the enhanced electrostatic repulsions among filaments leading to crystallization arise from deprotonation of COOH directly caused by x-ray irradiation (SOM text S11) (20). Hydroxide ions produced by water radiolysis may also contribute to the

enhanced ionization of carboxyl groups. Because of the high packing density of COOH on the surfaces of these extremely long filaments, a very small percentage of ionized COOH groups could potentially lead to a considerable increase in electrostatic repulsion among filaments and thus produce the observed ordering. Hydrated electrons produced by water radiolysis could also contribute to electrostatic repulsion through their addition to the C=O bonds (19). The charged species can return to their original structures through a previously proposed reconstitution mechanism (SOM text S11) (19, 20).

We have reported crystallization mediated by repulsive forces of high aspect ratio like-charge filaments within a network environment. We be-

lieve that arrangement of the filaments into aligned bundles trapped within a network is necessary for the observed crystallization and can be templated during growth of the very long filaments by self-assembly. The supramolecular nature and role of electrostatics in artificial systems studied here create a connection to cytoskeleton filaments, where similar crystalline bundles embedded in networks could occur naturally or be created by externally regulated charge density.

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- Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; G, Gly; K, Lys; R, Arg; T, Thr; and V, Val.
- This work was supported by the U.S. Department of Energy (grant no. DE-FG02-00ER45810). The SAXS experiments were performed at the DND-CAT located at Sector 5 of the APS. DND-CAT is supported by E. I. DuPont de Nemours and Company, Dow Chemical Company, and the state of Illinois. Use of the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract no. DE-AC02-06CH11357. Some additional support for this work was obtained from NIH National Institute of Dental and Craniofacial Research grant 5R01 DE015920 and NSF grant DMR-0605427. We thank the Biological Imaging Facility (BIF) at Northwestern for the use of TEM equipment.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1182340/DC1

Materials and Methods

SOM Text

Figs. S1 to S14

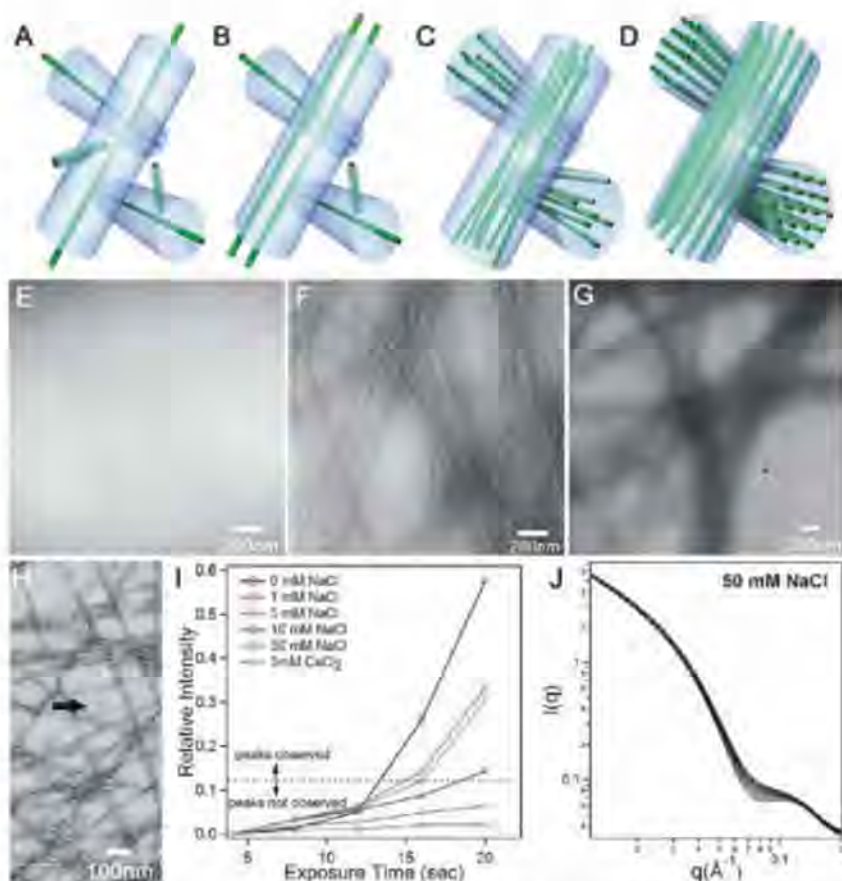
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**Fig. 4.** (A to D) Schematic illustration of the proposed templating model for filament bundle formation during self-assembly. (A) At early stages of self-assembly, interlocking of long filaments leads to the formation of a channel around each one of them. The long filament has many degrees of freedom within its channel but cannot leave it or allow others to enter its space. The volume of this imaginary channel should be determined mostly by the bulk density of long filaments. (B) Newly formed long filaments from smaller ones and from monomers are confined to grow within these channels. (C) Further growth causes the formation of bundles of aligned long filaments. (D) At high concentrations, hexagonally ordered filaments are spontaneously formed as a result of repulsive electrostatic forces and network confinement. Cryo-TEM image of filament bundles in 0.5 wt % solutions (E), 1 wt % solutions (F), and 2 wt % solutions (G). (H) Negatively stained TEM image of very early stages of self-assembly revealing the presence of small aggregates (marked with black arrow) coexisting with loosely packed long filaments at 0.1 wt % [this image offers support for the templating mechanism for bundle formation explained in (A) to (D)]. (I) The effect of added salts on x-ray-triggered crystallization of filaments; plot of the intensity of the primary Bragg peak as a function of exposure time to x-rays in 1 wt % solutions; when the intensity plotted is below the dotted line, the Bragg peak was not observed. (J) Set of 50 x-ray scattering profiles corresponding to a 1 wt % solution containing 50 mM concentration of NaCl obtained sequentially after 50 exposures of 4 s each to the x-ray beam (dose rate: 4806 Gy s<sup>-1</sup>).



# The Free-Energy Landscape of Clusters of Attractive Hard Spheres

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The study of clusters has provided a tangible link between local geometry and bulk condensed matter, but experiments have not yet systematically explored the thermodynamics of the smallest clusters. Here we present experimental measurements of the structures and free energies of colloidal clusters in which the particles act as hard spheres with short-range attractions. We found that highly symmetric clusters are strongly suppressed by rotational entropy, whereas the most stable clusters have anharmonic vibrational modes or extra bonds. Many of these clusters are subsets of close-packed lattices. As the number of particles increases from 6 to 10, we observe the emergence of a complex free-energy landscape with a small number of ground states and many local minima.

An isolated system of 10 interacting atoms or molecules will generally adopt a structure that differs in symmetry and average energy from that of a bulk liquid, solid, or even a system containing 100 particles. Yet the study of such small clusters has shed light on a wide variety of phenomena that are observed in the fields of condensed-matter physics and physical chemistry. Since Frank first predicted (1) that icosahedral short-range order would be a hallmark of liquid structure, the study of small-cluster geometry has provided key insights into the frustration underlying nonequilibrium phenomena such as nucleation and the glass transition (2–4). Experimental studies (5, 6) have confirmed this approach through the discovery of local cluster-like order in bulk liquids and glasses, with recent results (7) suggesting that structural arrest in condensed phases may be related to geometrical constraints at the scale of a few particles.

There remain many unresolved questions about cluster geometry and its connection to bulk behavior. Although experiments and simulations have determined the minimum potential-energy clusters for various interactions (8–10), the likelihood of observing a particular cluster structure depends on its free energy (11, 12). What cluster structures are favored by entropy? How does the competition between potential energy and entropy evolve as the number of particles  $N$  approaches the bulk limit? Experiments on atomic clusters have not systematically explored these questions; they are limited by short cluster lifetimes, non-equilibrium conditions, and the difficulties of obtaining real-space structures of individual clusters in free space (13).

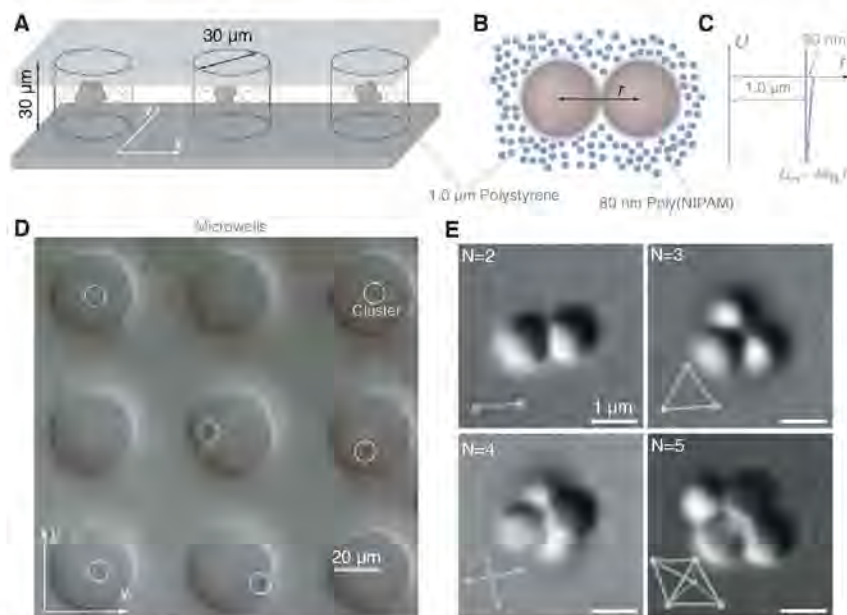
Here we report experimental results for the structures and free energies of small equilibrium clusters as a function of  $N$ , with  $N \leq 10$ . The experimental system is described in Fig. 1. We use colloidal particles rather than atoms, because

we can precisely control the interactions and directly observe the three-dimensional (3D) structures of the clusters using optical microscopy. To a good approximation, our particles act as “sticky” hard spheres, arguably the simplest nontrivial interaction that leads to clustering. The attraction arises from a depletion interaction with a range of about 1.05 times the particle diameter and a depth of about  $4k_B T$ , where  $k_B$  is Boltzmann’s constant and  $T$  is temperature. Because the pair potential is short-ranged, the total potential energy  $U$  of a given structure is well approximated by  $U = CU_m$ , where  $C$  is the number of contacts or depletion bonds and  $U_m$  is the depth of the pair potential (14). Although these particles form a gel

in bulk, the range and depth of the interaction are consistent with an equilibrium phase diagram showing a fluid-to-crystal transition (15).

We created clusters by isolating small numbers of polystyrene (PS) microspheres in cylindrical microwells filled with water and poly(*N*-isopropylacrylamide) (polyNIPAM) nanoparticles, which cause the depletion interaction. We chemically functionalized the microwells so that particles could not stick to the surfaces. This allows 3D clusters to form in the middle of the wells, unaffected by the boundaries. After the clusters reached equilibrium, we used optical microscopy to observe the cluster structures, and we collected statistics by scanning through the microwell plate, which contains thousands of isolated clusters. Although the number of particles per well is not controlled, we generated enough clusters at each value of  $N \leq 10$  to measure their occurrence frequencies. We then determined the free energies from the ensemble statistics through the Boltzmann distribution,  $\Delta F = -k_B T \ln P$ , where  $P$  is the probability of observing a given cluster.

We classified our clusters by comparing them to finite sphere packings. A previous theoretical study (16) enumerated the mechanically stable clusters of idealized hard spheres with infinitesimally short-ranged interactions, revealing the minima of the potential-energy landscape as a function of  $N$ . All of the minima at each value of  $N \leq 9$  have the same potential energy, which is a result not observed with longer-ranged potentials (17). We explored the structures and proba-



**Fig. 1.** (A) Diagram of experimental system (14). We used lithography to make microwells with depth and diameter of 30  $\mu\text{m}$  (see also fig. S1). These are filled with a suspension of 1.0- $\mu\text{m}$ -diameter PS spheres and 80-nm polyNIPAM microgel particles, which induce a depletion attraction as illustrated in (B). The number of PS particles per well varies, but the average is about 10. (C) Pair potential as estimated from the Vrij approximation to the Asakura-Oosawa potential (28, 29). Because the range of the depletion attraction is less than 1/10 of the PS sphere diameter, the interaction is strictly pairwise additive. (D) Optical micrograph of microwells with assembled colloidal clusters suspended inside. The circles highlight individual clusters in different microwells. There are about  $10^4$  microwells per slide. Scale bar, 20  $\mu\text{m}$ . (E) High-magnification optical micrographs of colloidal clusters in microwells with  $N = 2, 3, 4$ , and 5 particles. These are the only structures that form for  $N \leq 5$ . Scale bar, 1  $\mu\text{m}$ .

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bilities of these packings at finite temperature, which allows us to map the free-energy landscape (18, 19, 11). All of the observed cluster structures agree with the theoretical predictions. For example, for  $N < 6$ , we observed one unique structure for each  $N$ : a dimer for  $N = 2$ , trimer for  $N = 3$ , tetrahedron for  $N = 4$ , and triangular dipyrmaid for  $N = 5$ . The optical micrographs in Fig. 1 show the structures of the smallest clusters.

The first interesting case is  $N = 6$ . We observed two structures (Fig. 2 and fig. S2), both with  $C = 12$  contacts and therefore equivalent potential energy. The first is the octahedron, a Platonic solid. The second, we call a “polytetrahedron.” It consists of a triangular dipyrmaid with a third tetrahedron added to one of the faces. We observed transitions between the two states on time scales of minutes, indicating that the system is at equilibrium (movie S1).

Although these two structures have the same potential energies, the polytetrahedron occurs about 20 times more often than the octahedron, implying a free-energy difference of about  $3k_B T$ . This difference can be attributed only to entropy. As shown in Fig. 2, the measured probabilities for the two structures agree well with theoretical calculations based on standard approximations for the rotational and vibrational entropies in the classical limit (14).

The rotational entropy makes the largest contribution to the free-energy difference between the two structures (fig. S2). The rotational partition function is related to two geometrical quantities: the number of orientations, which is proportional to the moment of inertia, and the rotational symmetry of the cluster, or, alternatively, the number of ways one can assemble the same cluster by permuting particle labels (20). Formally, the ratio of the permutational degeneracies of two clusters is inversely proportional to the ratio of their symmetry numbers (21). This permutational degeneracy accounts for a factor of 12 in the polytetrahedron:octahedron probability ratio. The remaining factor of 2 comes from the differences in the moments of inertia and the vibrational entropies.

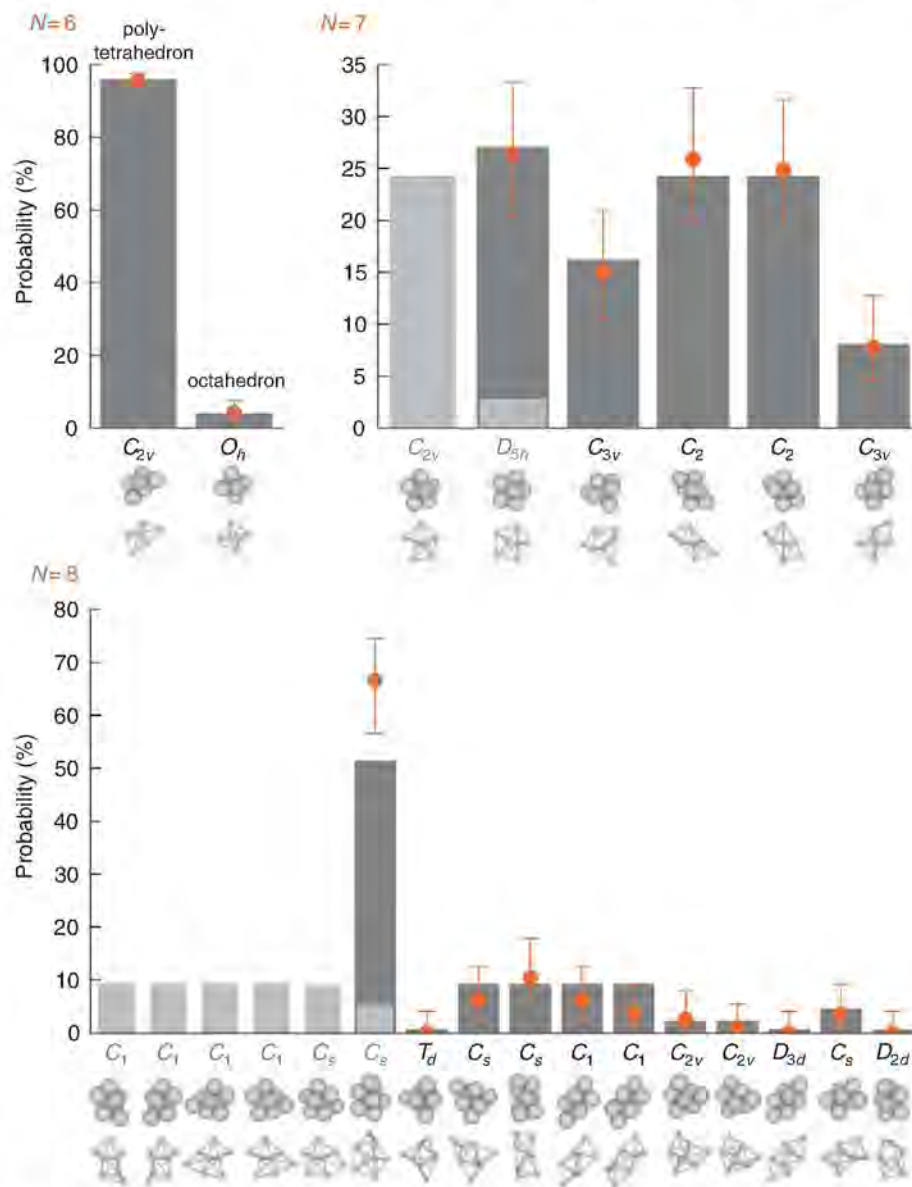
This result illustrates a general rule for clusters with short-range attractions: among clusters with the same potential energy, highly symmetric structures are extremely unfavorable at equilibrium. By contrast, for the longer-ranged Lennard-Jones 6-12 potential, the octahedron has lower potential energy than the polytetrahedron does (17), so that the dominant structure depends on temperature. The dominance of the polytetrahedron in our system may have consequences for nucleation; the equilibrium phase of attractive hard spheres is a face-centered cubic (FCC) crystal (15), which contains octahedral, not polytetrahedral, subunits.

At  $N = 7$ , the first chiral structures arise. We observed six cluster structures, two of which are chiral enantiomers. The experimental measurements agree well with the theoretical values for the probabilities of each structure (Fig. 2). For

these small clusters, the most pronounced influence on the probabilities comes from symmetry. At  $N = 8$ , 3 of the 16 different possible sphere packings never occur in the experiments. These three structures have the highest symmetry numbers,  $\sigma = 4, 6$ , and 12.

A few structures differ by such small changes in particle spacing that we cannot differentiate between them using our microscope. All of these are variants of pentagonal dipyrmaids. In a pentagonal dipyrmaid of seven spheres, the top and bottom spheres of the pyramid are separated by a small gap of  $\approx 0.05d$ , where  $d$  is the sphere diameter. If these two spheres are brought together, a gap of  $\approx 0.09d$  opens between two of the

spheres on the pentagon. Because we cannot resolve this gap in our experiments, we have binned these structures together at both  $N = 7$  and  $N = 8$ . The one statistically significant discrepancy between experiment and theory occurs at  $N = 8$ ; it arises because the experimental potential has a range that is comparable to the gap distance. Although we account for this extra potential energy in the probability calculations, the probabilities are sensitive to the magnitude of the potential at the gap distance. If the interaction energy differs from our estimated value by only  $0.1k_B T$  in the gap, the theoretical calculation falls within error of the experimental value. This difference could be due to polydispersity in either the depletant



**Fig. 2.** Comparison of experimental and theoretical (14) cluster probabilities  $P$  at  $N = 6, 7$ , and  $8$ . Structures that are difficult to differentiate experimentally have been binned together at  $N = 7$  and  $8$  to compare to theory. The calculated probabilities for the individual states are shown as light gray bars, and binned probabilities are dark gray. Orange dots indicate the experimental measurements, with 95% confidence intervals given by the error bars (14) (table S1). Renderings and point groups in Schönflies notation are shown for each structure. The number in the subscript of each symbol indicates the order of the highest rotational symmetry axis, and the letter indicates the symmetry group. The highest symmetry structures are those in  $D$ ,  $T$ , and  $O$  groups. Structures in  $C_1$  and  $C_2$  groups occur in chiral pairs.



or large spheres or to another interaction such as van der Waals forces.

The probability distributions in Fig. 2 are “field guides” to the free-energy landscapes at  $N = 6, 7$ , and  $8$ . Each structure represents a local free-energy minimum, the depth of which is proportional to the probability. We note two topographical features besides the trend toward structures with low symmetry: first, the number of local minima increases dramatically with  $N$ ; and second, the landscape is relatively flat for  $N = 7$  and  $8$ . In other words, there are many shallow minima, but no one minimum has a free energy much larger than any other.

The landscape undergoes a qualitative change for  $N \geq 9$ . Theoretically we expect some  $\Omega = 77$  structures at  $N = 9$  and  $\Omega = 393$  at  $N = 10$ , too many to catalog experimentally. We therefore measured only a subset of structures identified by our theoretical study (16). The subset we choose consists of clusters that fall into either of two categories: nonrigid structures, in which one of the vibrational modes is a large-amplitude, anharmonic shear mode; and structures with more than  $3N - 6$  bonds. Nonrigidity arises when a cluster contains half-octahedra that share at least one vertex, allowing the cluster to twist over a finite distance without breaking or forming another bond. We expect these packings to have high vibrational entropy. Structures with more than  $3N - 6$  bonds can occur for  $N \geq 10$ . These are the expected ground states.

Indeed, these special packings do occur with high frequency (Fig. 3 and table S2). Because most clusters at  $N = 9$  and  $10$  have equal potential energy, low symmetry, and therefore comparable rotational entropy, we expect the average probability of any one structure in a set of  $\Omega$  possible clusters to be of order  $1/\Omega$ . At  $N = 9$ , we expect an average probability of about 1%, and at  $N = 10$ , about 0.25%.

By contrast, the one nonrigid structure at  $N = 9$  occurs with  $P \approx 10\%$ . By using the theoretical  $\Omega$  and the experimental  $P$ , we estimate that the free energy of the nonrigid structure is about  $2k_B T$  lower than that of an average structure at  $N = 9$  (14). Thus the structure is highly stable by nearly half the free energy of an extra bond. The stabilization comes from the vibrational entropy associated with the nonrigid mode (movie S3). Our theoretical calculations (14) predict  $P \approx 3\%$ , which is lower than the observed probability but higher than all other clusters at  $N = 9$ . The discrepancy is due to the sensitive dependence of the vibrational partition function on the curvature of the pair potential near the minimum, a consequence of the nonrigid mode. A more precise calculation requires an accurate measurement of electrostatic effects in the experimental pair potential near the depletion well.

At  $N = 10$ , only 3 of the 393 theoretically possible clusters have  $3N - 5 = 25$  contacts, yet these occur about 10% of the time. Although we have only limited statistics for higher  $N$ , we con-

tinue to observe the prevalence of a few packings with  $3N - 5$  or more bonds. The structures with extra bonds have combined probabilities of 20 to 30% at  $N = 11$  and  $12$  (table S2). Again these probabilities are large compared to  $1/\Omega$ , even though, in several cases, the clusters have high symmetry. The potential-energy gain is therefore large enough to overcome the deficiency in rotational entropy.

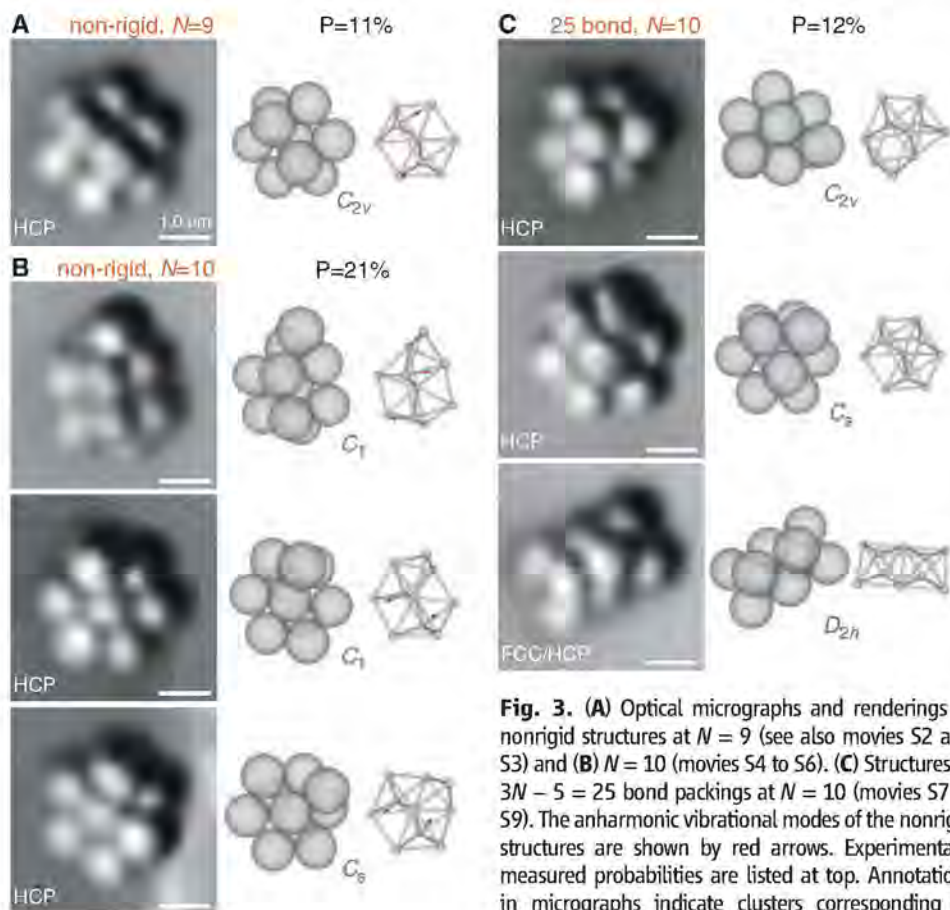
Perhaps the most striking feature of these clusters is that many are subsets of lattice packings, and in particular of the hexagonally close packed (HCP) lattice. The lattice packings are marked in Fig. 3. The underlying reason appears to be that both nonrigidity and extra bonds require the clusters to have octahedral subunits. The propensity for icosahedra (8, 22) in longer-range systems is absent in ours. We observed no icosahedra at either  $N = 12$  or  $13$ , presumably because neither 12-sphere or 13-sphere icosahedra are special clusters for short-range interactions; neither are nonrigid, neither have more than  $3N - 6$  bonds, and both have very high symmetry numbers ( $\sigma = 60$ ).

By using the same statistical mechanical approximations that were used to estimate probabilities for  $N \leq 8$ , we can calculate the free energies of all mechanically stable sphere packings that have been enumerated (16) up to  $N = 10$ . This yields the free-energy landscape shown in Fig. 4. We see that, in general, the locus of states is correlated with the rotational entropy, which is proportional to  $k_B \ln(\sqrt{I}/\sigma)$ , where  $I$  is the moment of inertia. The only states that lie below this locus occur at  $N = 9$  and  $10$ . These correspond to either nonrigid structures or structures with extra bonds, both of which appear as deeper minima.

The diagram also reveals some new features. First, low-symmetry polytetrahedral states proliferate as  $N$  increases. At  $N = 10$ , where clusters with extra bonds first appear, the absolute probability of observing these ground states is low because of the large number of low-symmetry states that lie at slightly higher free energy.

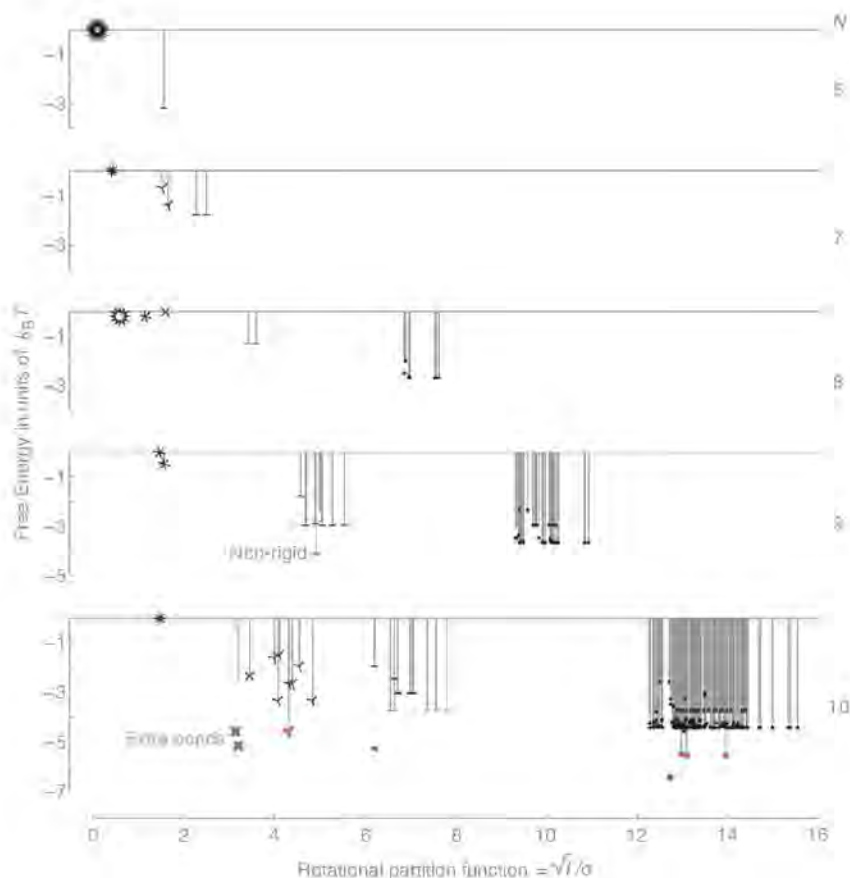
Second, the highest free-energy structures for  $N = 6$  to  $10$  are convex deltahedra (23), polyhedra with a long history in the field of condensed-matter physics (24, 3). These are not always the most symmetric structures; at  $N = 8$  the highest free-energy state is the deltahedron, a snub disphenoid that has lower symmetry than an eight-sphere tetrahedral cluster. The convex deltahedra also happen to be the same “minimal-moment” structures that are formed in capillary-driven assembly of colloidal particles (25). The optimal packings under these nonequilibrium conditions therefore correspond to the least-optimal packings at equilibrium.

Our results suggest that nucleation barriers and structural motifs in attractive hard-sphere systems such as colloidal suspensions will be different from those in systems with longer-range potentials, which tend to favor symmetric structures at sufficiently low temperatures. For  $N < 9$ , all of our clusters have nearly equivalent potential



**Fig. 3.** (A) Optical micrographs and renderings of nonrigid structures at  $N = 9$  (see also movies S2 and S3) and (B)  $N = 10$  (movies S4 to S6). (C) Structures of  $3N - 5 = 25$  bond packings at  $N = 10$  (movies S7 to S9). The anharmonic vibrational modes of the nonrigid structures are shown by red arrows. Experimentally measured probabilities are listed at top. Annotations in micrographs indicate clusters corresponding to subsets of HCP or FCC lattices. Scale bars, 1  $\mu\text{m}$ .





**Fig. 4.** Calculated minima of the free-energy landscape for  $6 \leq N \leq 10$  (14). The x axis is in units of the rotational partition function, where  $I$  is the moment of inertia (calculated for a particle mass equal to 1) and  $\sigma$  is the rotational symmetry number. The bond strength for the calculation is  $U_m = 4k_B T$ . Each black symbol represents the free energy of an individual cluster. The number of spokes in each symbol indicates the symmetry number (dot = 1, line segment = 2, and so on). Orange symbols are nonrigid structures, which first appear at  $N = 9$ , and violet symbols have extra bonds, first appearing at  $N = 10$ . Vertical gray lines indicate the contribution to the free energy due to rotational and vibrational entropy. The reference states are chosen to be the highest free-energy states at each value of  $N$ . The general trend is for low-symmetry states to be favored in proportion to their rotational entropy,  $k_B \ln(\sqrt{I}/\sigma)$ . The potential-energy contribution accounts for the vertical space between the violet symbols and their gray lines.

energy, and therefore the rotational entropy selects against symmetric structures at all temperatures. Specifically, the symmetry number and the permutational degeneracy have the greatest effect on the free energy; differences in moment of inertia do not contribute as much. Thus, even if cluster rotations are hindered, as they may be in a bulk supercooled liquid, the permutational degeneracy might still influence the probability of formation. At higher  $N$ , the most probable structures we observe involve combinations of octahedra and tetrahedra. Many of these structures are compatible with an HCP lattice, but not with FCC. Our results also suggest that the curvature of the pair potential near the minimum should affect nucleation, because the curvature determines the free energy of the nonrigid clusters.

Structures with fivefold symmetry, such as the pentagonal dipyrmaid and icosahedron, are highly unfavorable in our system. Therefore we do not expect icosahedra or other clusters with

fivefold symmetry to be a structural motif in attractive hard-sphere gels or fluid cluster phases (26), where the attraction is short-ranged.

We find that the most stable small clusters of hard spheres with short-ranged attractions can be determined by geometrical rules: (1) rotational entropy favors structures with fewer symmetry elements; (2) vibrational entropy favors nonrigid clusters, which have half-octahedral substructures sharing at least one vertex; and (3) potential energy favors clusters with both octahedral and tetrahedral substructures, allowing them to have extra bonds.

Our picture of the free-energy landscape is still incomplete. The qualitative features of the landscape are independent of temperature for our experimental system because the depletion interaction is fundamentally entropic (14). This will not be the case for other types of interactions, such as DNA-mediated attractions (27). Also, under nonequilibrium conditions, we expect a different distribution of structures than the ones

shown here. Finally, our model for the landscape does not account for energy barriers or the connectivity between minima. It will be interesting to see if further studies can explain the emergence of bulk crystallization or structural arrest in terms of these topographical features and their geometrical underpinnings.

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## Supporting Online Material

[www.sciencemag.org/cgi/content/full/327/5965/560/DC1](http://www.sciencemag.org/cgi/content/full/327/5965/560/DC1)  
Materials and Methods  
Figs. S1 and S2  
Tables S1 and S2  
Movies S1 to S9

28 August 2009; accepted 10 December 2009  
10.1126/science.1181263



# A Tricyclic Aromatic Isomer of Hexasilabenzene

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Benzene represents the showcase of Hückel aromaticity. The silicon analog, hexasilabenzene, has consequently been targeted for decades. We now report an intensely green isomer of  $\text{Si}_6\text{R}_6$  (R being 2,4,6-triisopropylphenyl) with a tricyclic structure in the solid state featuring silicon atoms with two, one, and no substituents outside the ring framework. The highly dispersed  $^{29}\text{Si}$  nuclear magnetic resonance shifts in solution ranging from +125 to -90 parts per million indicate an inhomogeneous electron distribution due to the dismutation of formal oxidation numbers as compared with that of benzene. Theoretical analysis reveals nonetheless the cyclic delocalization of six mobile electrons of the  $\pi$ -,  $\sigma$ - and non-bonding type across the central four-membered ring. For this alternative form of aromaticity, in principle applicable to many Hückel aromatic species, we propose the term dismutational aromaticity.

Benzene has stimulated synthetic and theoretical chemists since its discovery by Faraday in 1825 (1). It provided the basis for the famous rule associated with Hückel that the presence of  $4n + 2$  cyclically delocalized  $\pi$ -electrons implies aromaticity and related stabilization (2). Controversies regarding the concept continue to this day to provoke much productive research (3). Not surprisingly, the potential energy surface (PES) of  $\text{C}_6\text{H}_6$  is one of the best studied. More than 200 isomers have been identified, all being at least  $60 \text{ kcal mol}^{-1}$  higher in energy than benzene (4).

The prospect of aromaticity in compounds involving silicon was ignored for a long time because silicon was generally considered unable to form stable  $\pi$ -bonds (5). However, in 1981 West *et al.* reported a stable disilene, a compound with a Si-Si double bond sterically protected by bulky mesityl (2,4,6-trimethylphenyl) groups (6). Since then, about 100 disilenes (7) and even a few compounds with Si-Si triple bonds (8, 9) have been isolated, taking advantage of steric and/or electronic stabilization.

Cyclically delocalized  $\pi$ -systems involving silicon shifted into focus (10), and numerous breakthroughs have been achieved, such as the isolation of stable sila- and 1,2-disilabenzenes with planar, Hückel-aromatic structures (11, 12). Potentially aromatic compounds based on silicon alone are so far limited to persila-analogs of cyclopropenium cation (13) and cyclobutadiene dianion (14).

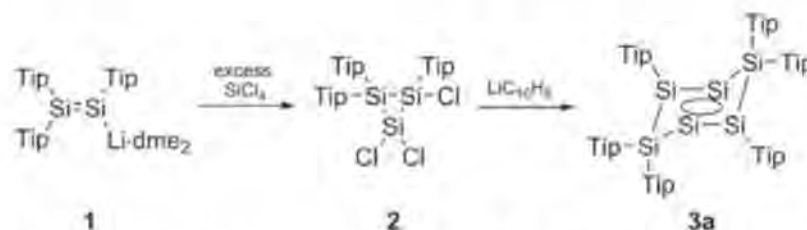
Because of the special role of benzene, a particularly enticing sila-aromatic compound is the elusive hexasilabenzene. After early predictions of a sixfold symmetry analogous to that of benzene (15), a chair-like confirmation resembling that of cyclohexane was established through theoretical calculations (16, 17). Experimentally, only an isomeric, nonaromatic hexasilaprismane

has been prepared by the reduction of a 1,1,2,2-tetrahalodisilane (18), suggesting that the choice of precursor may be important in any synthetic approach.

A way to prearrange half of the  $\text{Si}_6$ -scaffold became apparent to us in light of our previous results concerning the reaction of disilene 1 with an excess of dichlorosilanes affording cyclotrisilanes (19).

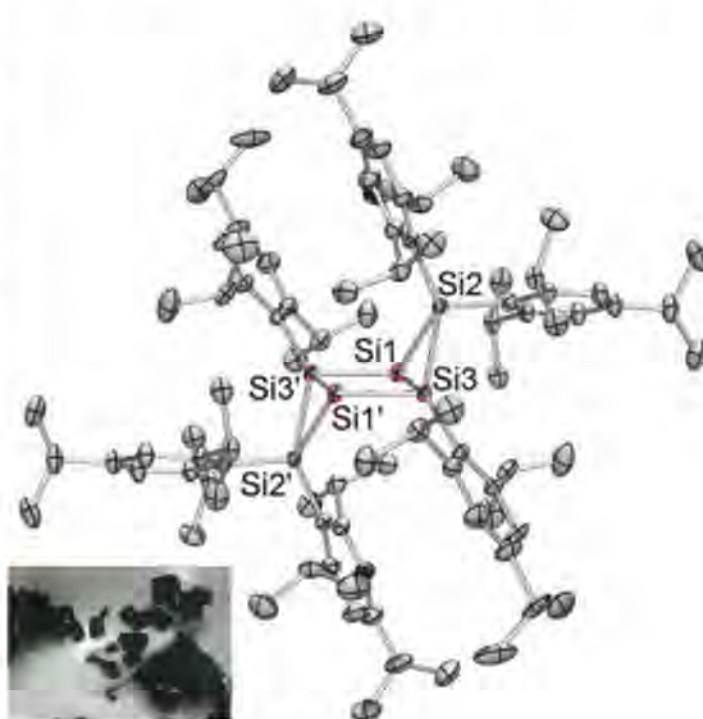
Extrapolating these results to silicon tetrachloride ( $\text{SiCl}_4$ ), we anticipated that cyclotrisilane 2 may become accessible, although the reaction of 1 with a shortage of  $\text{SiCl}_4$  yields a  $\text{Si}_5\text{Tip}_6$ -cluster (20). Conversely, rapid addition of an excess of  $\text{SiCl}_4$  to disilene 1 (21) afforded 2 in 59% isolated yield (Scheme 1) (22). Given the unsymmetric 1,1,2-substitution pattern of 2 (confirmed by means of x-ray diffraction) (figs. S2 to S4) (22), we expected it to provide an alternative entry to  $\text{Si}_6\text{R}_6$  chemistry. Reduction of 2 with three equivalents of lithium/naphthalene in tetrahydrofuran/diethylether (3:5 ratio) led to the product 3a (R being 2,4,6-triisopropylphenyl, or Tip), which was isolated as dark green crystals in 52% yield (Scheme 1) (22). The surprisingly stable 3a can be exposed to air for hours as a solid or for minutes in solution without detectable changes. Melting-associated decomposition was observed at  $216^\circ\text{C}$ .

The highest mass in the mass spectrum of 3a is observed at 1388 mass/charge ratio ( $m/z$ ) with the correct isotopic distribution for  $\text{Si}_6\text{C}_{90}\text{H}_{138}$  indicating the formation of a dimeric structure. On the basis of a two-dimensional  $^{29}\text{Si}/^1\text{H}$  nuclear magnetic resonance (NMR) correlation, the  $^{29}\text{Si}$  NMR signals at 124.6, -84.8, and -89.3 parts per million (ppm) were assigned to two silicon atoms each with one, two, and no directly attached Tip



**Scheme 1.** Synthesis of the dismutational hexasilabenzene isomer 3a (Tip, 2,4,6-triisopropylphenyl).

**Fig. 1.** Structure of 3a- $2\text{C}_{10}\text{H}_8$  (thermal ellipsoids at 50%). Hydrogen atoms and naphthalene molecules are omitted for clarity. (Insert) Photographic image of dark-green crystals of 3a- $\text{C}_6\text{H}_6$ . Selected bond lengths in angstroms (estimated SD) are Si1-Si2 = 2.3581(5), Si1-Si3 = 2.3275(5), Si1-Si3' = 2.3034(5), Si1-Si1' = 2.7287(7), and Si2-Si3 = 2.3375(5).



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substituent, respectively. The most positive  $^{29}\text{Si}$  NMR chemical shift is observed in the region typical for tetrasilyl-substituted  $\text{Si}=\text{Si}$  bonds (7) and far from the negative region where signals of tetracoordinate silicon atoms within small rings are found (18–20). This observation excluded any saturated structure as a possible product. The green color of **3a**, due to the longest wavelength absorption in the ultraviolet (UV)/vis spectrum at  $\lambda = 623$  nm, would also be highly atypical of saturated silicon compounds.

An x-ray diffraction study on single crystals of **3a** confirmed the features deduced from the spectroscopic data (Fig. 1) (22). The hexasilabenzene isomer **3a** crystallizes as a naphthalene solvate (**3a**·2  $\text{C}_{10}\text{H}_8$ ). Despite its tricyclic connectivity, the chair-like conformation of **3a** resembles the predicted puckered structure of hexasilabenzene (16, 17). **3a** exhibits a rhomboid  $\text{Si}_4$ -ring in the center ( $\text{Si1}$ ,  $\text{Si3}$  and symmetry equivalents  $\text{Si1}'$ ,  $\text{Si3}'$ ), with two opposing  $\text{SiTip}_2$  bridges ( $\text{Si2}$ ,  $\text{Si2}'$ ) pointing up- and downwards with respect to the  $\text{Si}_4$  plane [the angle to  $\text{Si1-Si2-Si3}$  is  $66.80(2)^\circ$ , with estimated SD in parentheses].  $\text{Si1}$  and  $\text{Si1}'$  show hemispherical coordination; all bonds point into one-half of an imaginary sphere, which is a common coordination in case of lead (23), tin, and germanium (24) but rare for neutral silicon compounds (20, 25). Despite the  $\text{SiTip}_2$  bridges,  $\text{Si1-Si3}$  [ $2.3275(5)$  Å] is only slightly longer than  $\text{Si1-Si3}'$  [ $2.3034(5)$  Å], both being at the shorter end of typical Si-Si single bonds. The rhomboidal distortion becomes apparent in one shorter diagonal distance [ $\text{Si1-Si1}'$   $2.7287(7)$  Å], which nonetheless is almost 17% longer than typical Si-Si single bonds.

On the basis of the analytical data, a resonance hybrid between **A** and **B** seemed best

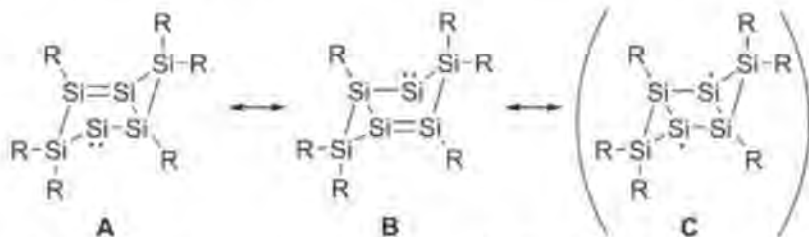
suited to describe the electronic structure of **3a** (Scheme 2). Contrasting the usual six  $\pi$ -electrons of Hückel aromatic systems, the mobile electrons in **3a** would nominally be of three different types. Two  $\pi$ -, two  $\sigma$ -, and two nonbonding electrons could be cyclically delocalized over four silicon centers with formal interruption of the  $\sigma$ -framework by two saturated  $\text{SiTip}_2$  homobridges (26). Because of the topological similarities to singlet diradicals of the Niece type (27), however, we initially did not exclude a certain diradical character in **3a** (resonance form **C**).

Density functional theory (DFT) calculations (22) on the Dip-substituted model compound **3b** (R being 2,6-diisopropylphenyl, or Dip) at the B3LYP/6-31G(d)-level of theory clarified the bonding situation (interactive table). The experimental geometry of **3a** is reasonably well reproduced despite substitution of the *para*-isopropyl groups with hydrogen atoms [for example, diagonal distance **3b**:  $\text{Si1-Si1}'$   $2.779$  Å (calculated),  $2.7287(7)$  (observed) Å]. The experimental trends in the  $^{29}\text{Si}$  NMR shifts of **3a** agree well with those calculated for the model compound **3b** [ $\delta = 170$ ,  $-46$ , and  $-83$  ppm, B3LYP/6-311+G(2df) basis on Si; 6-31G(d) on C,H]. Additionally, the UV/vis spectrum of **3b** simulated with time-dependent DFT (TDDFT) calculations is very similar to the experimental spectrum of **3a**. The closed-shell model **3b** appeared justified by the conformity of experiment and theory, which we lent further support by optimization of the triplet  $^3A_u$  state of **3b**, revealing a much longer diagonal distance ( $3.069$  Å) between the substituent-free silicon atoms. The adiabatic singlet-triplet gap of  $E_{S-T} = 24.1$  kcal mol $^{-1}$  and the dominant (>90%) contribution of a single closed-shell configuration to the complete active

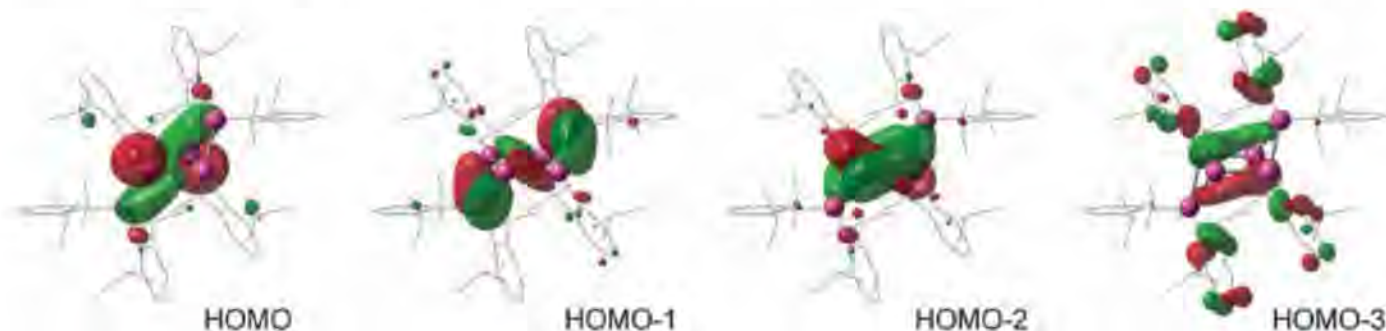
space self-consistent field (CASSCF) (8,8) computed multireference wave function for the parent model (**3c**, using the Si geometry computed for **3b**) supports a low diradical character for **3b** (28).

Inspection of the relevant molecular orbitals (MOs) of **3b** (Fig. 2 and interactive table) reveals that the highest occupied molecular orbital (HOMO) (orbital 309), HOMO-2, and HOMO-3 (the dismutative trio) are all cyclically delocalized around the central  $\text{Si}_4$  ring. The HOMO-3 is also conjugated with the aryl rings of four of the Dip groups (which is characteristic of  $\pi$ -aromatic bonds). In contrast, the HOMO-1 is clearly attributable to the larger  $\sigma$ -framework of **3b**, which includes the two bridging Si atoms and displays the typical appearance of an oligosilane system (29). The lowest unoccupied MO (LUMO) of **3b** is antibonding between the substituent-free silicon atoms, corresponding more closely to the diradical resonance form **C** (Scheme 2), which is in agreement with the longer distance between those atoms calculated for the  $^3A_u$  state of **3b**.

The topology of the total electron density in **3c** (R being Me) was initially probed by Bader's atoms-in-molecules (AIM) method (30). This analysis revealed bond critical points (BCPs) for both the annulated three-membered rings and for the central  $\text{Si}_4$ -motif but not for the long diagonal distance, corroborating the absence of direct bonding between  $\text{Si1}$  and  $\text{Si1}'$  (interactive table). The value of the electron density  $\rho(r)$  for BCP $_{\text{Si1-Si3}'}$  [ $0.078$  atomic unit (au)] was higher than for the homobond BCP $_{\text{Si1-Si3}}$  ( $0.073$ ), which hints at a cyclically delocalized through-bond interaction. An electron localization function (ELF) analysis (31) computed for the total electrons shows two disynaptic basins for  $\text{Si1-Si3/Si1'-Si3}'$  [ $1.42$  electrons ( $e$ ) each], two further disynaptic basins for  $\text{Si1-Si3'/Si1'-Si3}$  ( $1.96$   $e$ ) and two monosynaptic (nonbonding) basins located on  $\text{Si1}$  and  $\text{Si1}'$  ( $1.60$   $e$ ), for which resonance between forms **A** and **B** (Scheme 2) is a fair zero-order representation. The disynaptic basin centroids approximately correspond to the AIM BCPs. If the ELF is restricted to contributions from the top four MOs (dismutative trio plus HOMO-1), the same basin pattern is revealed [with further basins arising from the HOMO-1 (interactive table)]. The total-electron ELF isosurface includes a delocalized



**Scheme 2.** Resonance formulae (**A**) to (**C**) for **3a-c** [for **3a**, R = Tip; for **3b**, R = Dip (2,6-diisopropylphenyl); for **3c**, R = Me].



**Fig. 2.** HOMO, HOMO-1, HOMO-2, and HOMO-3 of model **3b** contoured at 0.035 atomic units (interactive table).



region around the Si<sub>4</sub> ring with a bifurcation threshold (0.755) similar to that reported for homoaromatic carbon rings (32). This feature is also present in the ELF isosurface, including just HOMO to HOMO-3. When the HOMO-1 is excluded, the ELF surface now starts to resemble the topology of the torus link computed using the  $\pi$ -MOs of benzene (33).

In order to quantify the aromaticity of **3a,b**, we calculated the nucleus-independent chemical shift, NICS(0), at the center of the Si<sub>4</sub> ring of **3b** (-23.8 ppm), which indicates substantial aromaticity (benzene  $\sim$ -10 ppm) but may also include shielding effects from the  $\sigma$ -framework (34). To estimate these latter effects, we computed the NICS(0) value for **4**, the hypothetical saturated hydrogenation product of **3b**. This in silico reduction has the effect of sequestering the two Si lone pair electrons and hence suppressing the dismutational resonance. The result (-6.4 ppm) suggests that the strongly diatropic NICS(0) value of **3b** is truly due to aromaticity. Further confirmation is obtained from the NICS(0) value of -3.3 ppm for the <sup>3</sup>A<sub>u</sub> triplet (and presumed non-aromatic) state of **3b** (resonance C) (Scheme 2).

In order to place **3b** in terms of relative energy, we calculated two of its isomers with Dip substituents: the experimentally known hexasilaprismane (**18**) and the hypothetical hexasilabenzenes. Both turned out to be lower in free energy  $\Delta G_{298}$  [B3LYP/6-31G(d); **3b**, 0.0; prismane, -11.7; benzene, -4.3 kcal mol<sup>-1</sup>] with the hexasilabenzenes surprisingly situated midway, which raises the intriguing possibility of a future synthesis of a stable hexasilabenzenes.

The general formalism leading to the type of aromaticity exemplified by **3a-c** is a twofold formal 1,2-shift of substituents in the classical

Hückel aromatic compounds: a double intramolecular dismutation. The formal oxidation states of the silicon atoms in **3a-c** are +2 (SiR<sub>2</sub>), +1 (SiR), and 0 (Si) as opposed to the uniform oxidation state of +1 in hexasilabenzenes. We propose the term dismutational aromaticity for a phenomenon that in principle should be applicable to any classical Hückel aromatic compound with at least six ring atoms.

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#### Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5965/564/DC1  
Materials and Methods

Figs. S1 to S6

Reference

Interactive Table

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## Combined Effects on Selectivity in Fe-Catalyzed Methylene Oxidation

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Methylene C-H bonds are among the most difficult chemical bonds to selectively functionalize because of their abundance in organic structures and inertness to most chemical reagents. Their selective oxidations in biosynthetic pathways underscore the power of such reactions for streamlining the synthesis of molecules with complex oxygenation patterns. We report that an iron catalyst can achieve methylene C-H bond oxidations in diverse natural-product settings with predictable and high chemo-, site-, and even diastereoselectivities. Electronic, steric, and stereoelectronic factors, which individually promote selectivity with this catalyst, are demonstrated to be powerful control elements when operating in combination in complex molecules. This small-molecule catalyst displays site selectivities complementary to those attained through enzymatic catalysis.

Methylene (secondary) C-H bonds are ubiquitous in organic structures and are often viewed by organic chemists as the inert scaffold upon which the traditional chemistry of "reactive" functional groups is performed. In contrast, the enzymatic oxidation of methylenes (i.e., C-H to C=O) is a fundamental transformation in biological systems and is crit-

ical for drug metabolism and the biosynthesis of secondary metabolites (1, 2). Selectivity in enzymatic catalysis is dictated by the local chemical environment of the enzyme active site, a feature that inherently limits substrate scope. Despite important advances in the discovery of catalysts for C-H oxidation, the ability to directly functionalize isolated, unactivated second-

ary C-H bonds with useful levels of selectivity in complex molecule settings under preparatively useful conditions (i.e., limiting amounts of substrate) has been restricted to the realm of enzymatic catalysis. A small-molecule catalyst capable of performing methylene oxidations with broad scope, predictable selectivities, and in preparatively useful yields would have a transformative effect on streamlining the practice of organic synthesis.

The paucity of methods for the oxidation of isolated, unactivated, and nonequivalent secondary C-H bonds underscores that they are, arguably, the most challenging chemical bonds to selectively functionalize. Reactivity for oxidizing such bonds had been observed with several catalysts, but generally in substrates where selectivity issues are circumvented (e.g., cyclohexane  $\rightarrow$  cyclohexanone) (3-11) or else where reactive sites are either electronically activated (i.e., adjacent

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to  $\pi$ -systems or heteroatoms) (12–15) or oriented favorably toward a directing group (16–19). The electron-rich nature of tertiary C–H bonds and their relative scarcity makes them more facile targets for selective oxidation (3–5, 19, 20). Bio-inspired catalysts with elaborate binding pockets have been viewed as the most promising candidates to effect site selectivity in unactivated, non-directed methylene oxidations (21).

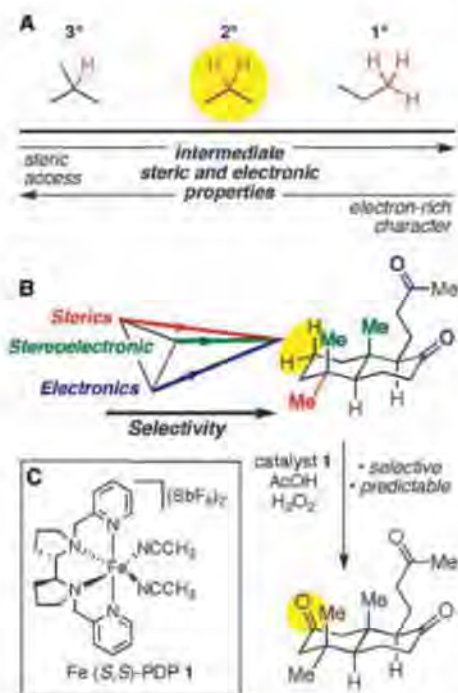
We recently reported a bulky, electrophilic catalyst [Fe(*S,S*-PDP) **1**] (Fig. 1C) (3, 4) that uses H<sub>2</sub>O<sub>2</sub> with acetic acid as an additive (22) to stereospecifically oxidize isolated, unactivated tertiary C–H bonds across a broad range of substrates. Surprisingly, predictably high levels of site selectivity were shown to be possible with **1** based on subtle steric and electronic differences among multiple tertiary C–H bonds. Encouraged by these results, we hypothesized that such a catalyst that is highly responsive to a combination of electronic and steric effects would be well suited for selective secondary C–H bond oxidation. Chemoselectivity advantages for secondary C–H bond oxidation may arise from increased steric accessibility compared to tertiary C–H bonds and greater electron-rich character compared to primary C–H bonds (Fig. 1A). Because secondary C–H bonds have intermediate electronic properties, they may be particularly

amenable to tuning by both electronic activation and deactivation. Moreover, since secondary C–H bonds are prevalent in ring systems, a variety of stereoelectronic effects (i.e., the influence of orientation of electron orbitals in space on chemical reactivity) may be readily exploited to achieve high site selectivity in their functionalization.

Here, we report that the same bulky, electrophilic Fe(*S,S*-PDP) **1** catalyst is capable of site-selective oxidation of isolated, unactivated secondary C–H bonds to afford mono-oxygenated products in preparatively useful yields—without the use of directing or activating groups. The site selectivities can be predicted in complex molecular settings on the basis of steric, electronic, and stereoelectronic rules derived from simple compounds. Whereas individually these effects in some cases afford modest selectivity, when manifest in combination in natural-product settings they result in useful levels of chemo-, site-, and even diastereoselective methylene oxidations (Fig. 1B). Moreover, because site selectivity in the case of small-molecule catalyst **1** is driven by its sensitivity to the local chemical environment of the substrate, we can achieve site

selectivities complementary to those obtained in biotransformations.

The highly electrophilic oxidant generated with (*S,S*)-**1** and H<sub>2</sub>O<sub>2</sub> allows for substantial electronic control over secondary C–H oxidation site selectivities (Fig. 2). *n*-Hexane, a hydrocarbon with no electronic biasing elements, is oxidized with no site selectivity (primary C–H bond oxidation has not been observed with **1**), forming ketones **2** and **3** in equal amounts (Fig. 2, entry 1) (23). The oxidation of methylene C–H bonds furnishes ketone products through alcohol intermediates via two stepwise, rapid oxidations (alcohols have been detected under conditions of limiting oxidant). A single electron-withdrawing group (EWG), such as a terminal ester, deactivates proximal sites via inductive effects, thereby introducing differential electronic environments that bias the site of oxidation. Specifically, high selectivity for ketone formation is observed at the most electron-rich methylene site (~50% yield), corresponding to that farthest from the EWG (entries 2 to 5). Consistent with our expectations (see above), the electronic biasing effect on methylene oxidations with (*S,S*)-**1** appears to be very strong; electronic deactivation by the ester



**Fig. 1.** (A) Chemical properties of aliphatic C–H bonds. (B) Synergistic effects on site selectivity. Steric, stereoelectronic (influence of orientation of electron orbitals in space on reactivity), and electronic influences on reactivity with catalyst **1** have an additive effect in complex molecule settings, which can lead to highly predictable and selective outcomes. (C) Electrophilic, bulky catalyst Fe(*S,S*-PDP) (**1**) for predictably selective aliphatic C–H oxidation.

**Fig. 2.** Electronic influences (inductive effects) on site selectivity in the oxidation of secondary C–H bonds of acyclic and cyclic substrates. An iterative addition protocol was used in which catalyst **1**, H<sub>2</sub>O<sub>2</sub> oxidant, and AcOH additive were added in three portions over a 30-min period to decrease the rate of bi- or multimolecular catalyst decomposition relative to substrate oxidation (3, 4, 23). Methylene C–H oxidation with **1** occurs preferentially at the methylene site most remote from EWGs; rsm, % recovered unoxidized starting material. The average mass balance for entries 2 to 13 is 94%.

(1 equiv.)  $\xrightarrow[\text{CH}_3\text{CN}, 25^\circ\text{C}, 30 \text{ min}]{(S,S)\text{-1 (15 mol\%)}^*, \text{AcOH (1.5 equiv.)}, \text{H}_2\text{O}_2 (3.6 \text{ equiv.})}$

| Entry | Major Oxidation Product Isolated % Yield <sup>†</sup> (%rsm) | Sites of Minor Oxidation % Yield |
|-------|--------------------------------------------------------------|----------------------------------|
| 1     |                                                              | —                                |
| 2     |                                                              | C4, 22%                          |
| 3     |                                                              | C5, 18%<br>C4, 14%               |
| 4     |                                                              | C4, 15%<br>C2, 1% <sup>‡</sup>   |
| 5     |                                                              | C4, 14%<br>C3, 9%                |
| 6     |                                                              | —                                |
| 7     |                                                              | C2, 14%                          |
| 8     |                                                              | C2, 2%                           |
| 9     |                                                              | C2, 8%                           |
| 10    |                                                              | C3, 25%                          |
| 11    |                                                              | C3, 24%                          |
| 12    |                                                              | C3, 28%                          |
| 13    |                                                              | C3, 37%                          |

<sup>†</sup> Iterative addition protocol. <sup>‡</sup> Unless otherwise noted, all compounds were isolated as single oxidized products. <sup>§</sup> GC yield. <sup>||</sup> Isolated in 52% yield as an inseparable 5:1 mixture of [C5:C3] oxidation products. <sup>¶</sup> Isolated in 69% yield as an inseparable 7:1 mixture of [C3:C2] ketones.



moiety in methylheptanoate **5** results in high selectivity for oxidation at the remote C-6 position relative to four other proximal secondary sites (entry 3, 51% yield). When tertiary sites are positioned  $\alpha$  or  $\beta$  to EWGs, the above trend still holds, highlighting the utility of this tactic for effecting the chemoselective oxidation of secondary C–H bonds in preference to tertiary C–H bonds (entries 4 and 5).

Electronic deactivation of proximal methylene sites also leads to predictably selective outcomes for oxidations within five- and seven-membered ring systems. In all cases examined, the major product is a result of ketonization at the methylene site most remote from the EWG (Fig. 2, entries 6 to 13). Consistent with selectivity trends found with acyclic substrates, cyclopentanone shows no conversion due to strong electronic deactivation of secondary sites  $\alpha$  or  $\beta$  to the carbonyl (entry 6). Increased product formation as high as 60% (**11**, entry 9) is observed as the EWG is rendered less inductively withdrawing and/or more remote from the ring (entries 7 to 9). Similar reactivity trends are observed with the oxidation of seven-membered rings; however, the benefits of an EWG on site selectivities are less pronounced (entries 10 to 13). For example, as the EWG is rendered less inductively withdrawing, the increased reactivity is offset by the erosion of site selectivity due to increased oxidation at the more proximal C3 position (entries 12 and 13). This trend reveals that a careful balance between substrate reactivity and selectivity must be struck when relying on electronic deactivation alone to achieve site selectivity with these simple substrates.

Examination of a series of six-membered cyclic substrates with EWGs demonstrated no preference for the more electronically favored C4 methylene site over the more proximal C3 site (Fig. 3A, entries 1 and 2). However, an increase in [C3:C4] site selectivity was observed with a sterically bulkier ring substituent that was no longer electronically deactivating (i.e., *tert*-butyl, entry 3). These trends suggest that stereoelectronic parameters based on conformational effects are strong contributing factors to the observed product distribution in six-membered ring oxidations with **1** (entries 1 to 3). In these cases, torsional strain between the bulky equatorial group and vicinal methylenes, generated by unfavorable 1,3-diaxial interactions at C1 and C3, is alleviated upon C3 C–H oxidation (24). In addition, such sterically bulky substituents also contribute to selectivity by inhibiting oxidation at adjacent sites. Methylene sites adjacent to bulky *t*-butyl or *gem*-dimethyl groups are disfavored in oxidations with (*S,S*)-**1**;  $\alpha$  positions are consistently oxidized in the lowest statistical yields (2% C2-ketone, entry 3; Fig. 3B, entry 4).

The bulky nature of the (*S,S*)-**1** catalyst even allows for subtle steric influences to have notable effects on the chemoselectivity of secondary versus tertiary C–H bond oxidations (Fig. 3B, entries 5 and 6). *cis*-Isomer **29**, containing

equatorial tertiary sites, undergoes preferential tertiary hydroxylation to provide alcohol **30** in 55% yield (tertiary:secondary = 4:1, entry 5). In contrast, oxidation of *trans*-isomer **33**, containing only axial tertiary sites, affords predominantly secondary C–H bond oxidation products **35** and **36** in a combined 50% yield (tertiary:secondary = 1:2, entry 6). Although a preference

for equatorial versus axial tertiary C–H bond oxidation has been noted for dioxirane oxidants (**5**), a reversal in chemoselectivity to favor secondary C–H bond oxidation has not been previously reported. This is most likely because the strong electronic preference for tertiary hydroxylation with an electrophilic oxidant can be overridden only with a very bulky oxidant

1 equiv.  $\xrightarrow[\text{CH}_3\text{CN}, 25^\circ\text{C}, 30 \text{ min}]{(S,S)\text{-1 (15 mol\%)}^*, \text{AcOH (1.5 equiv.)}, \text{H}_2\text{O}_2 (3.6 \text{ equiv.})}$

| Entry                                | Starting Material (rsm)                     | Oxidation Products % Yield <sup>†</sup>                                                                                |
|--------------------------------------|---------------------------------------------|------------------------------------------------------------------------------------------------------------------------|
| <b>A Stereoelectronic effects</b>    |                                             |                                                                                                                        |
| 1                                    | R = CO <sub>2</sub> Me, <b>16</b> , (35%)   | <b>17</b> <sup>‡</sup> , 43% <b>18</b> <sup>‡</sup> , 21%                                                              |
| 2                                    | R = CH <sub>2</sub> OPiv, <b>19</b> , (16%) | <b>20</b> <sup>‡</sup> , 46% <b>21</b> <sup>‡</sup> , 20%                                                              |
| 3                                    | <b>22</b> <sup>‡</sup> , (15%)              | <b>23</b> , 59% <b>24</b> , 12%                                                                                        |
| <b>B Steric Effects</b>              |                                             |                                                                                                                        |
| 4                                    | <b>25</b> , (15%) <sup>‡</sup>              | <b>26</b> , 21% <sup>‡</sup> <b>27</b> , 32% <sup>‡</sup> <b>28</b> , 17% <sup>‡</sup>                                 |
| 5                                    | <b>29</b> , (12%) <sup>‡</sup>              | <b>30</b> , 55% <sup>‡</sup> <b>31</b> , 9% <sup>‡</sup> <b>32</b> , 6% <sup>‡</sup><br>[tertiary : secondary] = 4:1   |
| 6                                    | <b>33</b> , (11%) <sup>‡</sup>              | <b>34</b> , 29% <sup>‡</sup> <b>35</b> , 22% <sup>‡</sup> <b>36</b> , 28% <sup>‡</sup><br>[tertiary : secondary] = 1:2 |
| <b>C Electron Activating Effects</b> |                                             |                                                                                                                        |
| 7                                    | <b>37</b> , (15%) <sup>‡</sup>              | <b>38</b> , 52% <sup>‡</sup> <b>39</b> , 6% <sup>‡</sup> <b>40</b> , 4% <sup>‡</sup>                                   |
| 8                                    | MeO <b>41</b> , 17%                         | MeO <b>42</b> , 62% <sup>‡</sup> + C6-ketone, 11%                                                                      |
| 9                                    | <b>43</b> , n = 1                           | <b>44</b> , n = 1, 41% <sup>‡</sup>                                                                                    |
| 10                                   | <b>45</b> , n = 2                           | <b>46</b> , n = 2, 47% <sup>‡</sup>                                                                                    |
| 11                                   | <b>47</b>                                   | <b>48</b> , 87% <sup>‡</sup>                                                                                           |

<sup>\*</sup> Iterative addition protocol. <sup>†</sup> All yields reported are isolated as pure products, unless otherwise noted. <sup>‡</sup> Isolated as an inseparable mixture of C3- and C4-ketones. <sup>§</sup> C2-ketone was observed in 2% GC yield. <sup>¶</sup> Yield determined by GC analysis.

**Fig. 3.** Stereoelectronic, steric, and electronic influences (hyperconjugative activation) on chemoselectivity and site selectivity in the oxidation of secondary C–H bonds in six-membered rings. **(A)** Alleviation of torsional ring strain in six-membered rings favors formation of C3- over C4-ketone products. Average mass balance for entries 1 to 3 is 89%. **(B)** Steric factors can lead to both site selectivity and chemoselectivity for secondary C–H bond oxidation in preference to tertiary C–H bonds with bulky catalyst **1**. The average mass balance for entries 4 to 6 is 86%. **(C)** Electron activation (via hyperconjugation) of aliphatic secondary C–H bonds for site-selective methylene oxidation; rsm, % recovered unoxidized starting material.

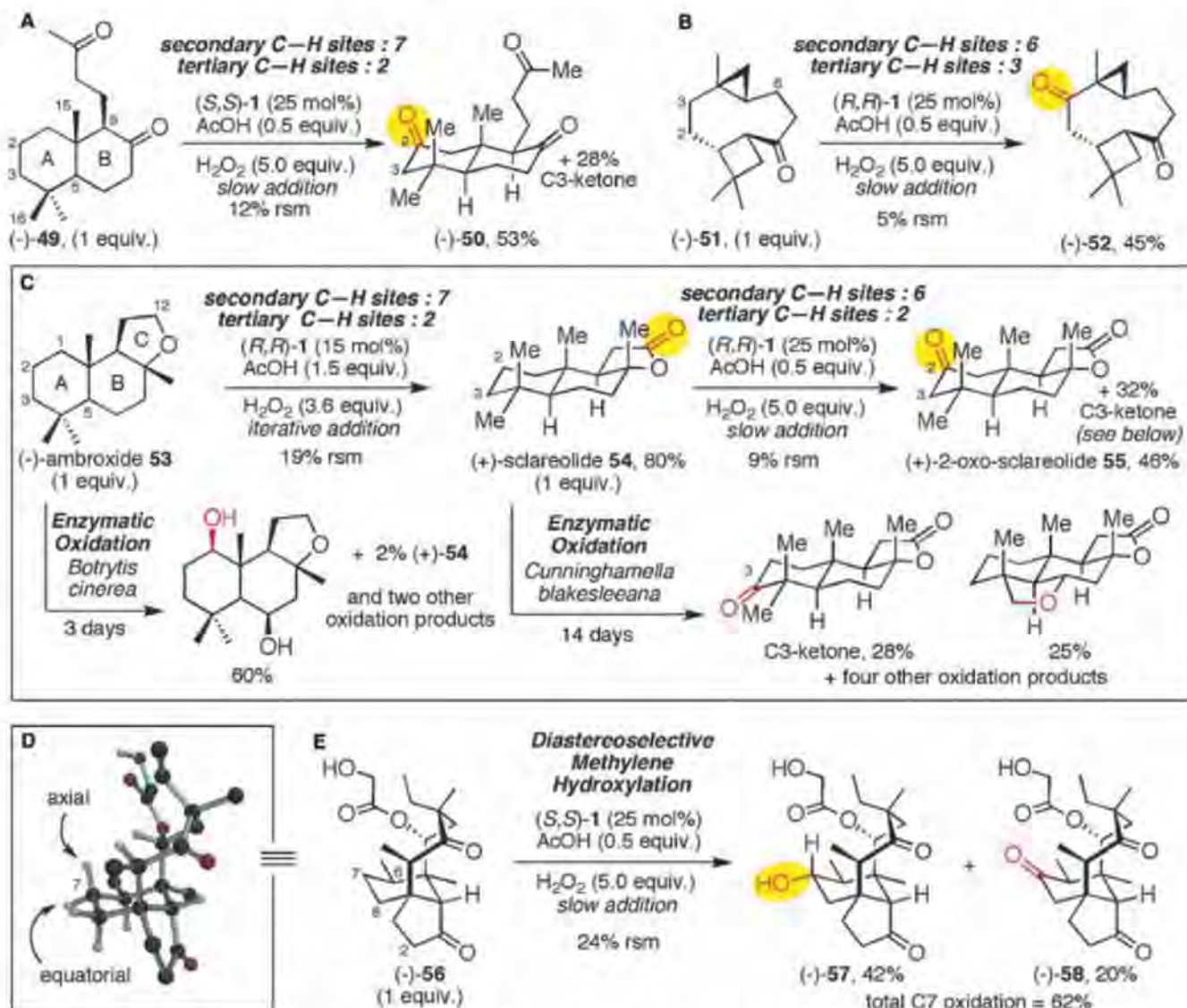


like that formed with catalyst *(S,S)*-1/H<sub>2</sub>O<sub>2</sub>. We hypothesized that the collective influence of these individual steric effects on site selectivity can have a combined effect on the selective oxidation of substrates with increased conformational rigidity (see below).

Although not required, electronic activating groups (EAGs) can be used to achieve site selectivities with catalyst **1** that are orthogonal to those based on inductive or steric effects (Fig. 3C). Groups capable of hyperconjugative activation, i.e., donation of electron density from a filled orbital to the antibonding orbital of a C–H bond, may be used to activate adjacent C–H bonds toward oxidation. The strained cyclopropane ring, having appreciable p-character in the C–C

bonding orbitals, can activate the cyclopropylcarbinyl methylenes through hyperconjugation (25). For example, cyclopropanes can direct secondary C–H bond oxidations at sites that are sterically or inductively deactivated. Although quaternary alkyl substitution diverts oxidation away from  $\alpha$ -hydrogens on a cyclohexane ring (Fig. 3B, entry 4), selective oxidation at these sites occurs when a cyclopropyl group is installed (Fig. 3C, entry 7). The site selectivity in the oxidation of methylheptanoate can also be altered to favor the C5 position (disfavored based on inductive effects: Fig. 2, entry 3, C5:C6 = 1:3) via the incorporation of a cyclopropyl moiety (Fig. 3C, entry 8, C5:C6 = 6:1). Following the previously established electronic trends,

the more electron-rich of the two methylene sites flanking the cyclopropyl ring is still selectively oxidized. No oxidation of tertiary C–H bonds on the cyclopropyl ring was observed. Another powerful form of hyperconjugative activation is the interaction of lone-pair electrons of an etheral oxygen with adjacent C–H bonds (26, 27). This electronic activating effect enables the selective oxidation of five- and six-membered cyclic ethers into lactone products (entries 9 and 10). Lower product yields and poor mass balance in these cases can be attributed to overoxidation of the open-chain hydroxyaldehyde form of the intermediate lactol. Consistent with this explanation, when a seven-membered cyclic ether is oxidized, lactone is not observed but adipic acid (**48**) is



**Fig. 4.** Additive effects of steric, electronic, and stereoelectronic factors on the selectivity of secondary C–H bond oxidations of terpenoids. A slow addition protocol was used in which catalyst **1** and H<sub>2</sub>O<sub>2</sub> oxidant were added via syringe pump over a 1-hour period to decrease the rate of bi- or multi-molecular catalyst decomposition relative to substrate oxidation (4, 23). (A) Electronic and steric deactivation, as well as stereoelectronic activation via alleviation of 1,3-diaxial six-membered ring interactions, lead to predictable and highly selective oxidations by **1** on manool-derived dione (-)-49. (B) Cyclopropane-induced methylene activation (via hyperconjugative elec-

tron activation), as well as electronic deactivation, leads to selective oxidation of (-)-51. (C) Transformation of (-)-ambroxide (**53**) into (+)-55 via sequential, intermolecular secondary C–H oxidation. Microbial biotransformations of (-)-53 and (+)-54 demonstrate site selectivities orthogonal to those of Fe(*R,R*-PDP) **1**. For a complete list of all the oxidation products formed in the microbial oxidations, see figs. S1 and S2 (23). (D) Energy-minimized structure of (-)-dihydropleuromutilone (**56**). (E) Diastereoselective methylene hydroxylation of (-)-56; rsm, % recovered unoxidized starting material.



isolated in 87% yield (entry 11). In more conformationally rigid substrates, overoxidation is not observed (see below). Benzylic sites are also activated toward selective oxidation, although this effect is limited by the requirement for EWG substitution on the aromatic ring to prevent its oxidation (compound **51**) (23).

Having established a framework of selectivity rules for catalyst **1** with relatively simple molecules, we sought to apply the system to complex natural products. In this respect, we sought to mimic the action of selective oxidative enzymes like the cytochrome P-450s. Terpenoids, the largest and most diverse class of natural products, are biosynthesized through pathways that introduce oxygen functionality independent of C–C bond-forming events. Topologically diverse hydrocarbon skeletons are forged through a sequence of carbonyl condensations, reductions that remove postcondensation oxygen functionality, and cyclizations (28). Oxidation patterns are subsequently installed via late-stage oxidative tailoring enzyme modifications (1). We hypothesized that the individual electronic, steric, and stereoelectronic effects on selectivity in oxidations of simple compounds with catalyst **1** (see above) would be heightened when combined in complex molecule settings.

We first examined the oxidation of dione (–)-**49**, a molecule derived from the diterpenoid (+)-manool, that comprises a substituted *trans*-decalin core and contains a total of 28 C–H bonds, 14 of which are secondary and two tertiary (Fig. 4A). According to our selectivity rules, we hypothesized that the two tertiary sites should be electronically and sterically deactivated toward oxidation: C9 is  $\alpha$  to a ketone, and C5 is both in an axial orientation and adjacent to a *gem*-dimethyl group. With regards to methylene oxidation, we recognized that the two ketones electronically deactivate the side chain and the entire B ring, leaving the secondary C–H bonds of the A ring as the most likely sites for oxidation. The most distal site from the sterically bulky quaternary centers on the A ring is C2; moreover, repulsive 1,3-diaxial interactions with two methyl substituents (C15 and C16) may be relieved by C2 C–H bond oxidation. Despite nine possible sites of oxidation, we observed oxidation at only the C2 and C3 sites. Consistent with our analysis, C2 is the major site of oxidation affording (–)-**50** in 53% isolated yield (12% recovered starting material, rsm). This example highlights the capacity for distinct selectivity factors to become mutually reinforcing in a complex molecule setting, thereby producing predictable and highly selective oxidation of secondary C–H bonds.

Site selectivity through hyperconjugative activation is demonstrated with the Fe(PDP)-catalyzed oxidation of the terpenoid derivative (–)-**51**, a compound with a sensitive carbogenic framework composed of a cyclopropane- and cyclobutane-annulated nine-membered ring (Fig. 4B). We hypothesized that the cyclopropane moiety would effectively override steric effects

and activate the two adjacent methylene groups, with C3 being favored for oxidation relative to C6 because of its remoteness from the ketone moiety. Upon exposure of (–)-**51** to catalyst **1**, a number of minor, unidentified oxidation products were observed; however, oxidation at C3 occurred preferentially to furnish ketone (–)-**52** as the major product in a preparatively useful 45% yield (5% rsm). This oxidation proceeds in higher yield and selectivity with (*R,R*)-**1** than (*S,S*)-**1**, which we rationalize as a better matching of catalyst geometry with substrate topology.

Selective, sequential oxidations (27) may be achieved with an electrophilic oxidant by engaging an electron-activating group, in an otherwise electronically unbiased hydrocarbon skeleton, to direct the initial site of oxidation. Upon oxidation, the newly installed ketone, acting as an EWG, will inductively bias the molecule during further oxidation. The two-step oxidative transformation of (–)-ambroxide (**53**) into (+)-2-oxo-sclareolide (**55**) demonstrates the power of this strategy to effect highly selective sequential oxidations with (*R,R*)-**1** (Fig. 4C). The first methylene oxidation exploits the activated C–H bonds on the five-membered cyclic ether (C ring) to achieve high site selectivity at one site among nine other possible sites of oxidation. Oxidation of the  $\alpha$ -ethereal C–H bonds furnishes (+)-3*R*-sclareolide (**54**) in 80% yield, even amid numerous other electronically and sterically accessible secondary and tertiary sites of oxidation. The newly installed lactone of (+)-**54** now serves as an EWG to inductively deactivate the B and C rings, making the secondary C–H bonds on the A ring most favorable toward further oxidation. As noted above with an analogous *trans*-decalin structure [(–)-**49**], C2 and C3 are the only notable sites of oxidation with catalyst **1**/H<sub>2</sub>O<sub>2</sub>. Among these two sites, C2 is favored on the basis of destabilizing diaxial interactions and results in (+)-2-oxo-sclareolide (**55**) being the major oxidation product in 46% isolated yield (9% rsm). Oxidation of (–)-**53** with catalyst **1** afforded very little yield of (+)-**55**, even at higher catalyst and oxidant loadings. This observation suggests that rapid catalyst decomposition may play a role in preventing overoxidations with this mild oxidant (4). Through an interplay of electronic activation and deactivation, opposite hemispheres of a complex molecule can be selectively oxidized through intermolecular oxidation steps with a single catalyst. Oxidations of (–)-**53** and (+)-**54** with microbial enzymes, which are reported to furnish some of the oxidation products we observe with catalyst **1**, require substantially longer reaction times (3 to 14 days), provide (+)-**54** from (–)-**53** in only minimal yield (<5%), and demonstrate no observable formation of (+)-**55** from (+)-**54** (Fig. 4C) (29, 30). These examples illustrate that site-selective oxidations can be predictably achieved with catalyst **1** that are often complementary to those accessible via enzymatic oxidations.

Pleuromutilin and its derivatives have attracted extensive attention from both the pharmaceutical and academic communities because of their potent activities against drug-resistant Gram-positive bacteria and their unusual tricyclic diterpenoid structure. Despite potent antibacterial activities, their hydrophobic nature limits their solubility and promotes rapid degradation by cytochrome P-450 at the C2 and C8 positions, resulting in inactive metabolites (31). On the basis of our findings with (–)-**53** and (+)-**54**, we anticipated that catalyst (*S,S*)-**1** would selectively oxidize at an alternative site, providing a distinct functional group handle for further chemical modification. High levels of regio-, chemo-, and stereoselectivity were observed in the oxidation of pleuromutilin derivative dihydropleuromutilone [(–)-**56**] with (*S,S*)-**1**/H<sub>2</sub>O<sub>2</sub> (Fig. 4, D and E). The C14 glycolic acid side chain, a prevalent site for chemical modification, remained intact during oxidation. This result illustrates the extraordinary chemoselectivity possible with this oxidant; even a primary alcohol can be electronically deactivated from oxidation when  $\alpha$  to a carbonyl. Electron-withdrawing carbonyl and ester moieties effectively deactivate the five- and eight-membered rings toward electrophilic oxidation with (*S,S*)-**1**/H<sub>2</sub>O<sub>2</sub>, rendering the six-membered ring as the mostly likely region for oxidation. Although the axial tertiary C–H bond at C6 is electronically accessible, it is situated in the sterically congested cavity of the five- and six-membered fused ring system (Fig. 4D). We hypothesized that the C7 methylene is the single most electron-rich and sterically accessible site for oxidation by catalyst (*S,S*)-**1**/H<sub>2</sub>O<sub>2</sub>. Consistent with this analysis, we observed that 62% of the starting material is oxidized specifically at C7 (24% rsm) among 11 possible sites of oxidation. The major oxidation product is the result of diastereoselective hydroxylation at the more sterically accessible equatorial secondary C–H bond, providing (–)-**57** as a single isomer in 42% isolated yield (Fig. 4E). In comparison, ketone at C7 [(–)-**58**] is provided in only 20% yield. Although the C–H bond of a secondary alcohol is highly electronically activated toward oxidation, energy minimization of (–)-**56** shows that this bond is sterically deactivated by virtue of its proximity to the eight-membered ring (Fig. 4D).

A small-molecule reagent sensitive to the local chemical environment of the substrate has enabled selective methylene C–H oxidations that are predictable using the fundamental concepts of electronics, sterics, and stereoelectronics. We anticipate that these findings will contribute to redefining how C–H bonds are viewed in synthetic planning, future methods development, and the exploration of molecular diversity.

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35. Dedicated to Professor Eric N. Jacobsen on his 50th birthday for his inspirational work on selective oxidations. We gratefully acknowledge R. J. Pakula for performing gas chromatography experiments toward elucidating the steric effects on oxidation site selectivity; M. A. Bigi, D. J. Covell, and E. M. Stang for helpful discussions and checking our spectroscopic data; and S. A. Reed for helpful discussions and checking our experimental procedure. M.S.C. was a Harvard University graduate student who completed his doctoral work with M.C.W. at the University of Illinois at Urbana-Champaign. This work was submitted to M.S.C.'s committee as part of his thesis on 28 August 2009 and was presented by M.C.W. at the Welch Symposium on 27 October 2009. We are grateful to the A. P. Sloan Foundation, the Camille and Henry Dreyfus Foundation, Bristol-Myers Squibb, Pfizer, Abbott, and the University of Illinois for financial support. M.S.C. is a 2008 Bristol-Myers Squibb Graduate Fellow in Synthetic Organic Chemistry. A U.S. patent on "Selective Aliphatic C-H Oxidation" is pending (application 12/245,086).

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5965/566/DC1

Materials and Methods

References

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## A Basal Alvarezsauroid Theropod from the Early Late Jurassic of Xinjiang, China

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The fossil record of Jurassic theropod dinosaurs closely related to birds remains poor. A new theropod from the earliest Late Jurassic of western China represents the earliest diverging member of the enigmatic theropod group Alvarezsauroida and confirms that this group is a basal member of Maniraptora, the clade containing birds and their closest theropod relatives. It extends the fossil record of Alvarezsauroida by 63 million years and provides evidence for maniraptorans earlier in the fossil record than *Archaeopteryx*. The new taxon confirms extreme morphological convergence between birds and derived alvarezsauroids and illuminates incipient stages of the highly modified alvarezsauroid forelimb.

The presence of the basal avialan (*1-3*) *Archaeopteryx* in the latest Late Jurassic (Tithonian) and the poor fossil representation of more basal maniraptoran taxa in contemporaneous or slightly older deposits indicate either a gap in the stratigraphic record or, more controversially, that birds are not related to theropods (*4*). Recent discoveries of Middle-Late Jurassic maniraptorans (*5-7*) from China are starting to fill in the temporal gap, but the ages of these new taxa are poorly resolved (*8, 9*), and they do not clarify basal maniraptoran diversification because very little character evidence separates them from birds (*5, 7*).

Here, we describe a three-dimensionally preserved, nearly complete skeleton of an alvarezsauroid theropod, *Haplocheirus sollers*, gen. et spec. nov. (*10*), from orange mudstone beds in the upper part of the Shishugou Formation in Wucuiwan area, Junggar Basin, Xinjiang, China. Radiometric dating constrains the age of this fossil to between  $158.7 \pm 0.3$  and  $161.2 \pm 0.2$  Ma (million years ago) (*11*), corresponding to the Oxfordian marine stage in the early Late Jurassic (*12*). The dates for *Haplocheirus* reduce the conflict between the fossil record and phylogenetic hypotheses that early maniraptoran diversification took place in the Jurassic. Alvarezsauroids are known from South America (*13-15*), Asia (*16-19*), North America (*20, 21*), and Europe (*22, 23*). The basal phylogenetic position and early temporal position of *Haplocheirus* imply that Alvarezsauroida originated in Asia rather than South America (*14, 20*). Derived members of the Alvarezsaur-

oidea were originally thought to be flightless basal avialans (*18*) because they share many morphological characteristics with birds, including a loosely sutured skull, a keeled sternum, fused wrist elements, and a posteriorly directed pubis. *Haplocheirus* preserves plesiomorphic morphological characteristics that confirm a basal position for Alvarezsauroida within Maniraptora (*24*), demonstrating that these features of derived alvarezsauroids represent dramatic convergences with birds.

IVPP (Institute of Vertebrate Paleontology and Paleoanthropology) V15988 (Figs. 1 and 2 and figs. S4 to S9) is ~140 cm in total body length as preserved but is missing the end of the tail (estimated total length 190 to 230 cm; table S1). The bones of the braincase are coossified and the neurocentral sutures are visible, suggesting that the animal is likely a young adult or late-stage subadult.

The gracile, low skull (Fig. 2A and figs. S4 to S7) is well preserved in three dimensions. The narrow, elongate rostrum becomes taller and wider just anterior to the large, anterolaterally facing orbits. The dorsoventrally thin jugal is triradiate, unlike the rodlike jugal of more derived alvarezsauroids (*17*). In lateral view, the basisphenoid is oriented at 45° to horizontal, a condition present in some troodontids (*25*) and in alvarezsauroids (*17*). The basiptyergoid processes are long and project ventrolaterally, a morphology known only in primitive birds and alvarezsauroids among Theropoda (*17*).

At least 30 small maxillary teeth are present in *Haplocheirus*, as in *Shuvuuia* (*17*), *Pelecanimimus* (*26*), therizinosauroids (*27*), and troodontids (*28*). Unlike the conical, unserrated teeth of *Mononykus* (*28*) and *Shuvuuia* (*29*), however, the maxillary teeth of *Haplocheirus* are

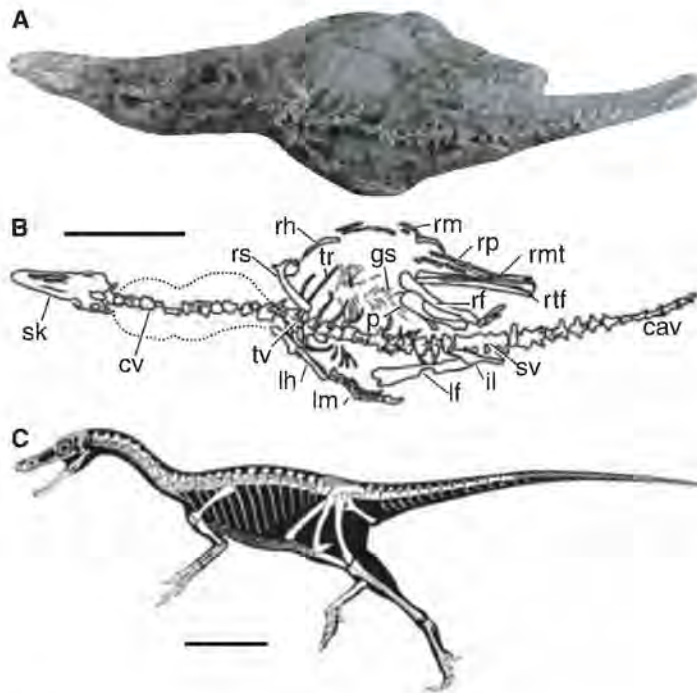
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**Fig. 1.** *Haplocheirus sollers* (IVPP V15988).

(A) Photograph of holotype in dorsal view. (B) Line drawing of holotype in dorsal view. Dashed line indicates crocodyli-form fossil underlying cervical vertebrae. (C) Reconstruction. Gray fill indicates portions of the skeleton that are not preserved. Abbreviations: cav, caudal vertebrae; cv, cervical vertebrae; gs, gastralia; il, left ilium; lf, left femur; lh, left humerus; lm, left manus; p, pubis; rf, right femur; rh, right humerus; rm, right manus; rmt, right metatarsus; rp, right pes; rs, right scapula; rtf, right tibia and fibula; sk, skull; sv, sacral vertebrae; tr, thoracic ribs; tv, thoracic vertebrae. Scale bar: 25 cm.



recurved and bear small serrations posteriorly (fig. S4). *Haplocheirus* shows marked heterodonty in both the maxilla and the dentary, with the maxillary teeth diminishing in size posteriorly (fig. S5) and the lower jaw bearing large, subconical, unserrated anterior teeth and smaller, recurved, serrated posterior teeth (fig. S4).

The cervical centra lack the strong opisthocoealous condition of *Shuvuuia* and *Mononykus* (29). The five sacral vertebrae all lack the ventral keel present in other alvarezsauroids (29). The centrum of sacral five is amphiplatyan, whereas in *Mononykus* and *Shuvuuia* (29) the last sacral centrum bears a convex posterior surface for articulation with the procoelous first caudal vertebra. Similar to other alvarezsauroids, the mid-caudal vertebrae have short and anterodorsally oriented prezygapophyses, whereas in most coelurosaurs the prezygapophyses of the mid-caudal vertebrae are long and horizontally oriented. A sternum was not found with the specimen.

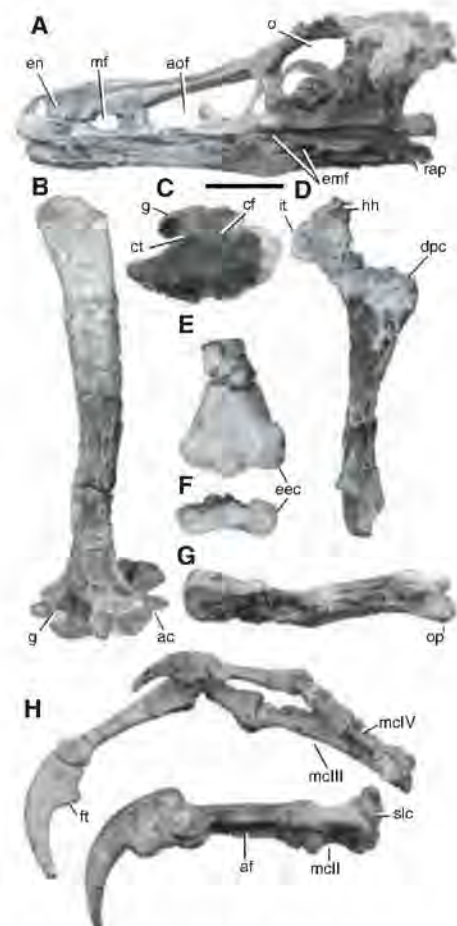
The ovate coracoid has a long posteroventral process and bears a well-developed biceps tubercle, which is lost in other alvarezsauroids. Similar to the basal alvarezsauroid *Patagonykus* (30), the hypertrophied rectangular internal tuberosity of the humerus is separated from the humeral head by a distinct notch, and the humeral ectepicondyle is large and hemispherical distally. The incomplete ulna bears a well-developed olecranon process, as do all alvarezsauroids. Proximally, the radius articulates with a lateral concavity on the ulna.

Unlike the avianlike carpometacarpus (29) of more derived alvarezsauroids, the distal carpals are unfused. Metacarpal II (the medial metacarpal) is 1.9 times the width of metacarpal III (Fig. 3), wider than in most maniraptorans but narrower

than in *Mononykus* and *Shuvuuia* (29). Metacarpal IV is only half the length of metacarpal III, a condition unknown in other theropod dinosaurs. The middle digit is the longest in the manus, unlike in *Shuvuuia*, where the second and third digits are both much shorter than the first digit (31). Manual phalanx II-1 bears a deep axial furrow ventrally, as in all alvarezsauroids (14). The manual unguals are curved and bear distally placed flexor tubercles, unlike the unusual unguals of derived alvarezsauroids (31), which are flat and lack flexor tubercles.

The pubis projects anteroventrally (figs. S8 and S9), which is the plesiomorphic condition for maniraptorans, in contrast with the vertical pubes of *Patagonykus* (30) and highly retroverted pubes of derived alvarezsauroids (29). The curved femur bears a plesiomorphic alariform lesser trochanter, consistent with the femoral morphology of *Alvarezsaurus* (14) and *Patagonykus* (30). The lateral distal condyle of the femur is conical (fig. S9) and projects further distally than the medial condyle, a morphology known only in some troodontids and alvarezsauroids (14). There is no development of an avianlike medial cnemial crest, as in *Mononykus* and *Shuvuuia* (26). The third metatarsal is fully exposed in anterior view and is a component of the ankle joint, as in the basal alvarezsauroids *Patagonykus* and *Alvarezsaurus* (13, 30).

A phylogenetic analysis of 99 theropod taxa shows that *Haplocheirus* is the basalmost member of the Alvarezsauroida [Fig. 3 and fig. S1; see Supporting Online Material (SOM) for full details]. *Haplocheirus* possesses unambiguous synapomorphies that support the monophyly of the Alvarezsauroida, including long basipterygoid processes, a vertically inclined basisphenoid,



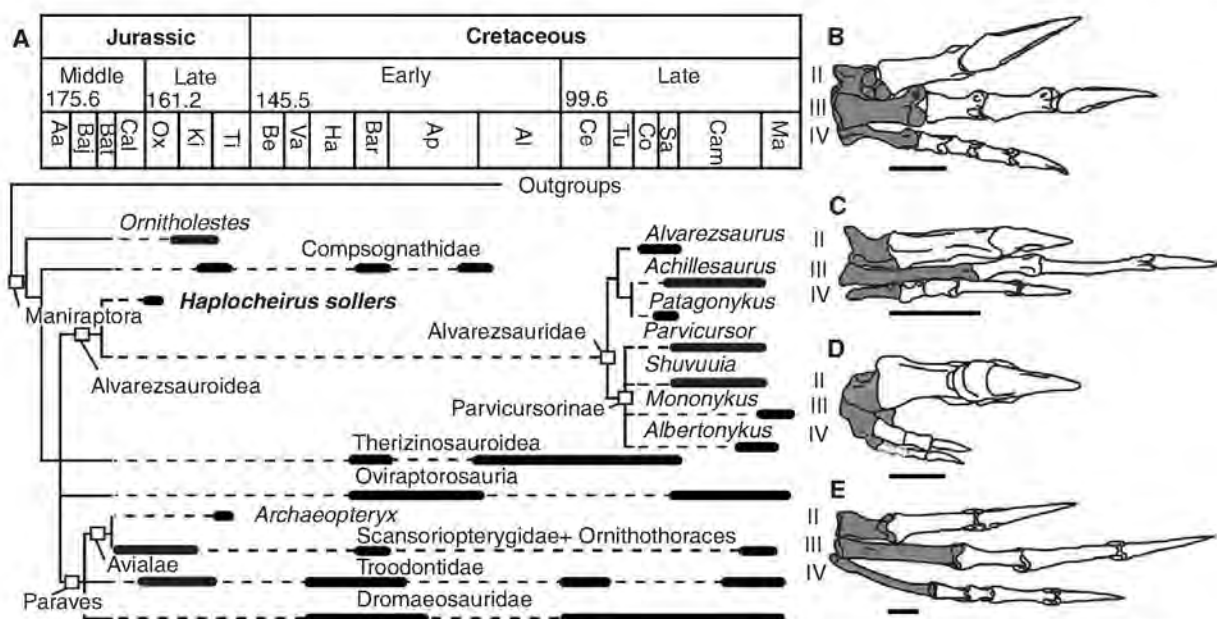
**Fig. 2.** *Haplocheirus sollers* (IVPP V15988) skull and forelimb elements. (A) Skull in left lateral view. (B) Right scapula in lateral view. (C) Right coracoid in lateral view. (D) Right humerus in lateral view. (E) Left distal humerus in flexor view. (F) Left distal humerus in distal view. (G) Right ulna in medial view. (H) Left manus in lateral view, with digit II separated from manus. Abbreviations: ac, acromion process; af, axial furrow; aof, antorbital fenestra; cf, coracoid foramen; ct, coracoid tuberosity; dpc, deltopectoral crest; eec, ectepicondyle of humerus; emf, external mandibular fenestrae; en, external naris; ft, flexor tubercle; g, glenoid; hh, humeral head; it, internal tuberosity; mclI to mclIV, metacarpals II to IV; mf, maxillary fenestra; o, orbit; op, olecranon process of the ulna; rap, retroarticular process; slc, semilunate carpal. Scale bar: 4 cm.

a hypertrophied internal tuberosity of the humerus proximally level with the humeral head, a large humeral ectepicondyle, a pronounced axial furrow on the flexor surface of manual phalanx II-1, and a conical lateral distal condyle of the femur.

Although a basal position of alvarezsauroids within Maniraptora has been proposed (24, 32–34), the global phylogenetic placement of this group within Theropoda has been untested by the recent description of new alvarezsauroid material from Canada (20), Argentina (15), and Mongolia (19). Early analyses placed alvarezsauroids as either sister to, or nested within, Avialae (28, 29). Alvarezsauroida has also been hypothesized as



**Fig. 3.** Phylogenetic relationships of *Haplocheirus* *sollers* and hand comparisons of selected theropods. (A) Simplified, temporally calibrated cladogram of the strict consensus of 69 most parsimonious trees produced in this analysis (see also fig. S1). Thick lines with rounded end caps indicate stratigraphic range of known taxa. Thin black lines with square ends indicate phylogenetic relationship. Dashed lines represent ghost lineages implied by the stratigraphic distribution of fossils with respect to the phylogenetic relationships shown here (note the exceptionally long ghost lineage for Alvarezsauroidea). Arabic numerals indicate ages in millions of years before present. Boxes represent named clades. (B to E) Reconstructed theropod mani scaled to digit two length. (B) *Allosaurus fragilis* after Madsen (40). (C) *Haplocheirus sollers* (IVPP V15988). (D) *Shuvuuia deserti* after Suzuki et al. (31). (E) *Deinonychus antirrhopus* after Ostrom (41). Metacarpal elements are shaded, dashed lines



(note the exceptionally long ghost lineage for Alvarezsauroidea). Arabic numerals indicate ages in millions of years before present. Boxes represent named clades. (B to E) Reconstructed theropod mani scaled to digit two length. (B) *Allosaurus fragilis* after Madsen (40). (C) *Haplocheirus sollers* (IVPP V15988). (D) *Shuvuuia deserti* after Suzuki et al. (31). (E) *Deinonychus antirrhopus* after Ostrom (41). Metacarpal elements are shaded, dashed lines

in (D) indicate inferred digital elements. Abbreviations: II to IV, manual digits II to IV; Aa, Aalenian; Baj, Bajocian; Bat, Bathonian; Cal, Callovian; Ox, Oxfordian; K, Kimmeridgian; Ti, Tithonian; Be, Berriasian; Va, Valanginian; Ha, Hauterivian; Bar, Barremian; Ap, Aptian; Al, Albion; Ce, Cenomanian; Tu, Turonian; Co, Coniacian; Sa, Santonian; Cam, Campanian; Ma, Maastrichtian. Scale bar: 5 cm (B), 4 cm (C and E), 0.5 cm (D).

the sister taxon to Ornithomimosauria (33). Our phylogenetic analysis agrees with still other research (2, 24) in placing Alvarezsauroidea as a relatively basal maniraptoran group. Our most parsimonious trees do not support either avialan or ornithomimosaurian affinities for the Alvarezsauroidea, although the ornithomimosaur hypothesis is nearly as well supported. The shortest tree found when constraining the Alvarezsauroidea to be sister to or nested within Avialae is seven steps longer than the optimal tree. The minimum length of trees produced when constraining Alvarezsauroidea to be sister to or nested within Ornithomimosauria is one step longer than the optimal tree (see SOM for phylogenetic details).

The Middle to Late Jurassic (Bathonian-Kimmeridgian) age (8, 9) of newly described maniraptorans from China (6, 7) implies that the Alvarezsauroidea have a minimum ghost lineage (35) of ~63 million years (Fig. 3). The presence of *Haplocheirus* in the early Late Jurassic of China confirms the prediction made from this ghost lineage that alvarezsaurids were present at this time. To explore the effect of *Haplocheirus* on congruence between stratigraphic information and phylogenetic hypotheses for the Coelurosauria (36–39), we compared the gaps in the fossil record implied before and after inclusion of *Haplocheirus* (37, 39). The addition of the earliest Late Jurassic *Haplocheirus* improves the stratigraphic fit of the maniraptoran fossil record to the topologies recovered in our analysis by an average of 14%, from a mean fit of 0.41 to 0.48 (fig. S3).

*Haplocheirus* is the largest alvarezsaurid known from complete material (see SOM), and its

basal phylogenetic position suggests a pattern of miniaturization for the Alvarezsauroidea, relatively rare in dinosaurs but convergently evolved in Paraves (2). Derived alvarezsaurids have a simplified, homogeneous dentition convergent with that of some extant insectivorous mammals (20), but *Haplocheirus* has recurved, serrated teeth and caniniforms that suggest carnivory was the primitive condition for the clade. The presence in *Haplocheirus* of only slightly reduced second and third manual digits and curved unguals with flexor tubercles on these digits implies that the hand was fully functional and *Haplocheirus* retained some grasping ability, unlike the presumably limited function of the greatly reduced lateral manual elements of *Mononykus* and *Shuvuuia* (31). The mediolaterally narrow McIII (metacarpal three) and the greatly shortened and slender McIV suggest that the extensive digital reduction and fusion seen in derived alvarezsaurids was already under way by the earliest Late Jurassic, proceeded from lateral to medial on the manus and, surprisingly, initially involved reduction in length of only McIV.

#### References and Notes

- We use the definition of Avialae from (2), the least inclusive clade containing *Archaeopteryx lithographica* and crown group birds, rather than the definition from (3), all taxa sharing a more recent common ancestor with *Archaeopteryx* than with *Troodon formosus*.
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## Supporting Online Material

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References

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# Anciently Asexual Bdelloid Rotifers Escape Lethal Fungal Parasites by Drying Up and Blowing Away

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Asexuality has major theoretical advantages over sexual reproduction. An important evolutionary puzzle, therefore, is why exclusively asexual metazoan lineages rarely endure. The Red Queen hypothesis posits that asexuality is rapidly extinguished by relentlessly coevolving parasites and pathogens. If so, any long-lasting asexual lineage must have unusual alternative mechanisms to deal with these biotic enemies. Bdelloid rotifers are freshwater invertebrates that abandoned sexual reproduction millions of years ago. Here, we show that cultured populations of bdelloids can rid themselves of a deadly fungal parasite through complete desiccation (anhydrobiosis) and disperse by wind to establish new populations in its absence. In Red Queen models, spatiotemporal escape can decouple and protect asexuals from coevolving enemies. Thus, our results may help to explain the persistence of the anciently asexual Bdelloidea.

**S**exual reproduction reduces the efficiency of gene transmission by up to 50%, disrupts favorable gene combinations, spreads disease, and is energetically expensive (1). Yet, paradoxically, sex is nearly ubiquitous: Obligate asexuality occurs in less than 1% of animal species (1, 2), and its scattered distribution at the tips of phylogenetic trees implies that abandoning sex condemns a clade to extinction before it can radiate sufficiently to achieve high taxonomic rank.

The class Bdelloidea (phylum Rotifera) is a famous exception. During three centuries of observation, more than 450 species of these tiny freshwater invertebrates have been described, but

neither males nor meiotic eggs have ever been recorded (3). Molecular evidence supports the inference that bdelloid rotifers have been obligately asexual for tens of millions of years (2–5).

Hypotheses for sexual reproduction suggest that it is maintained because it removes deleterious mutations, facilitates coevolution with parasites and pathogens, or both (2, 5–8). However, mutational hypotheses have been challenged empirically (1, 5, 6, 9), and mutational load may be less problematic than predicted, because the bdelloids have persisted despite accumulating mutations faster than related sexual clades (4). In contrast, the coevolution (Red Queen) hypothesis has received considerable empirical and theoretical support (1, 5–11).

Under Red Queen models, biotic interactions favor sex by relentlessly imposing fluctuating, time-lagged, frequency-dependent selection (6–10). However, asexuality can be maintained

in one special case: when vulnerable hosts can temporarily shed locally coadapted parasites and pathogens and disperse without them to uninfected habitats (11). Migration and clonal diversity at the population level can then substitute for recombination and genetic diversity at the individual level, allowing mobile hosts to avoid the costs of sex while continuing to “outrun” their enemies (11, 12). Dispersal further favors asexuality by reducing intergenerational transmission of infections (13).

Three unusual characteristics of bdelloid rotifers suggest that this scenario may apply to them (5, 11, 12). First, bdelloids can survive extended (up to 9 years) and repeated bouts of complete desiccation (anhydrobiosis) at any life stage (14, 15). Second, anhydrobiotic bdelloids have an extraordinary potential for wind dispersal as tiny (usually <300 μm) ovoid propagules (called “tuns”) (14–17), resulting in circumglobal distribution of some taxa (18). Third, bdelloids can thrive in almost any moist habitat, rapidly colonizing even the most ephemeral patches of moss or rainwater on every continent (14–18).

All identified parasites of bdelloid rotifers are oomycetes or hyphomycete fungi. Most belong to *Rotiferophthora*, a genus of obligate, lethal fungal endoparasites that are exclusive to bdelloids (19, 20). Infections spread when rotifers ingest spores (conidia), which lodge in their pharynx and produce assimilative hyphae. As the rotifer is killed and digested, hyphae puncture its integument and, at the air/water interface, produce conidiophores carrying hundreds of new conidia.

We investigated whether populations of the bdelloid rotifer *Habrotrocha elusa* can escape the fungal parasite *Rotiferophthora angustispora* (19) in space and time, through anhydrobiosis and subsequent wind dispersal. We transferred rotifers from a monoclonal population singly to

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Petri dishes (20). A control group was allowed to proliferate without parasites, whereas six experimental groups were inoculated after 9 days with  $\sim 712 (\pm 210 \text{ SD})$  conidia of *R. angustispora*. The first experimental group remained hydrated throughout the experiment, but the remaining five were desiccated 72 hours after inoculation, maintained at 39.8% relative humidity ( $\pm 1.8\%$  SD) for 7, 14, 21, 28, and 35 days, respectively, and then rehydrated.

Without anhydrobiosis, *R. angustispora* exterminated *H. elusa* populations in  $13.8 \pm 2.6$  days (mean  $\pm$  SD,  $n = 12$  populations), during which time the uninfected control populations were peaking (Fig. 1A). In the dishes that were rehydrated after 7 days of desiccation, the parasite initially seemed absent. Recovered rotifers were healthy, desiccation had fractured and scattered the fungal conidiophores, and water samples were not infectious to fresh rotifer cultures, suggesting absence of viable conidia (20). However, within 48 hours, hyphae began to re-emerge from the remains of dead rotifers, generating new conidiophores and exterminating the populations in  $18.6 \pm 4.0$  days (Fig. 1B). Excluding a four-day lag while the fungus regenerated, the time course of the resurgent infection in the rehydrated populations did not differ from that observed in the hydrated, inoculated group (i.e., complete extermination in  $14.6 \pm 4.0$  days versus  $13.8 \pm 2.6$  days; unpaired  $t$  test,  $n = 22$ ,  $t = 0.544$ ,  $P = 0.59$ ). Identical fungal regeneration was observed after 14 days of desiccation (with extermination in  $14.7 \pm 2.3$  days).

However, dramatically different results occurred in the other three experimental groups. After 21 days of anhydrobiosis, 60% of rotifer populations remained free of fungal infections for the duration of the experiment (20 weeks). Longer periods of desiccation were even more effective: After 28 and 35 days, 85 and 90.5%, respectively, of rehydrated populations remained fungus-free and thus grew significantly more (Fig. 1C).

In nature, desiccated bdelloids are dispersed by wind, adding a second dimension in which they might evade parasites (16). To simulate wind dispersal, we established a heavily infected population of *H. elusa* in a dish with a natural, friable substrate (sterilized moss and silt) and placed it in a wind chamber with a turbulent flow equivalent to a light breeze (20). Desiccated substrate particles were blown toward empty target dishes 30 to 40 cm away. After 7 days in the chamber, these “wind dispersal” dishes had accumulated  $5.01 \text{ mg} (\pm 2.52 \text{ SD})$  of material; they were then hydrated and monitored for 6 weeks.

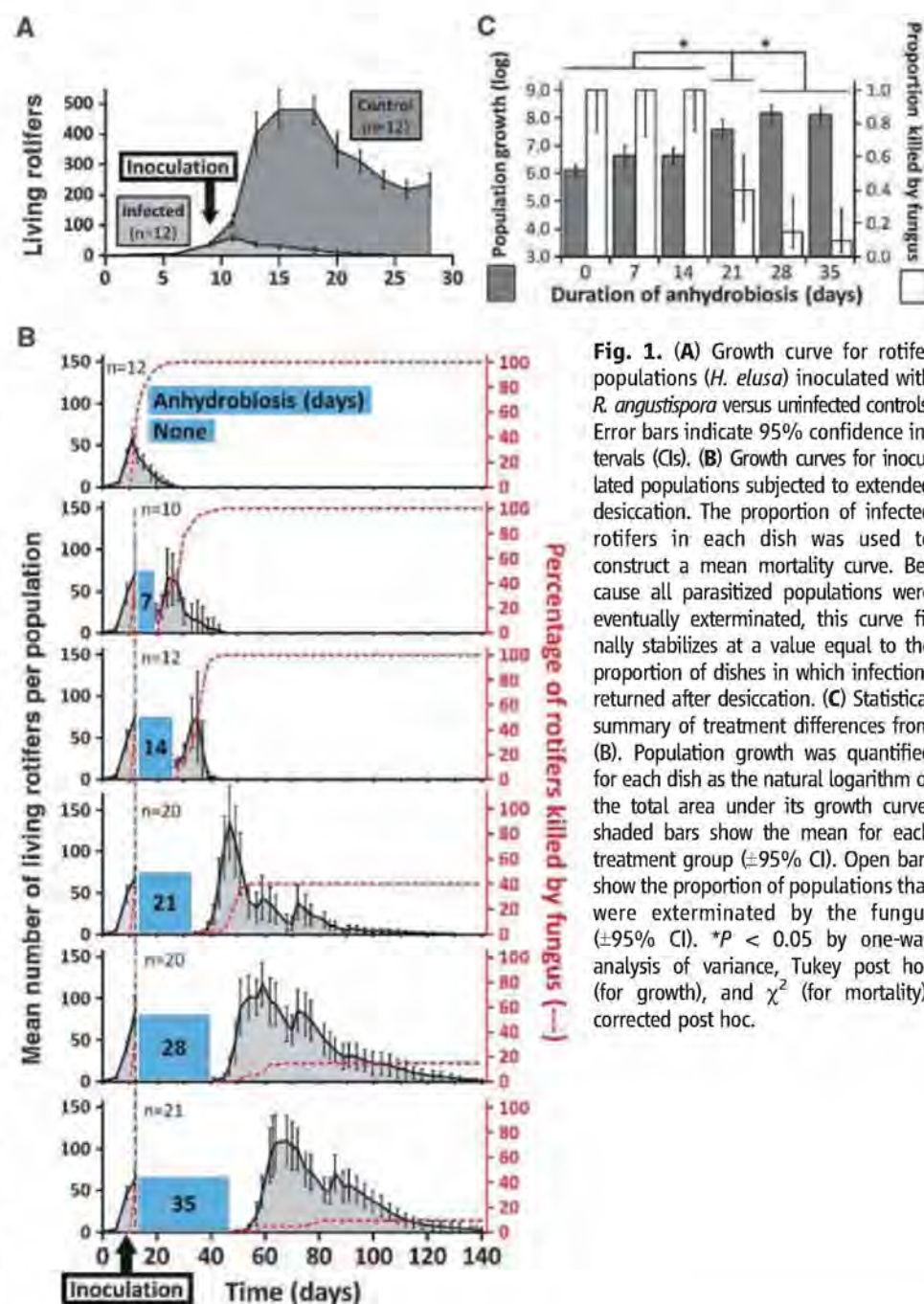
Enough rotifers survived to establish populations in 17 of 24 target dishes. Fungal infections appeared in seven of the newly established populations and exterminated them within  $16.4 \pm 5.0$  days (Fig. 2A). However, the 10 remaining wind-dispersed populations (58.8%) never exhibited infections. We replicated this experiment and obtained similar results (i.e., 63.6% of wind-dispersed populations remained fungus-free; see

fig. S2). For comparison, “wet dispersal” dishes ( $n = 24$ ) were created by pipetting 5.0 mg of well-mixed, suspended substrate from an identical infected source dish that was not desiccated (20). Rotifers established populations in all dishes, but fungal conidia also were readily transmitted, and all populations were exterminated in  $22.3 \pm 5.7$  days (Fig. 2B).

Our first experiment indicates that anhydrobiosis allows *H. elusa* to shed *R. angustispora*, apparently because the fungus is less resistant to extended desiccation than its host (Fig. 1C and fig. S1). Our second experiment demonstrates that wind dispersal acts in concert with anhydrobiosis to facilitate escape from *Rotiferophthora*, which is primarily waterborne. Three weeks of in situ desiccation (Fig. 1B) were required to

achieve the same rate of parasite elimination ( $\sim 60\%$ ) that was seen after only 7 days in the wind chamber (Fig. 2 and fig. S2). In nature, dispersal distances and maximal durations of anhydrobiosis are certainly far greater (14–18, 20). Together, our results demonstrate that anhydrobiosis, even for relatively short periods, enables this bdelloid rotifer to disperse without accompaniment by a lethally coadapted fungal parasite.

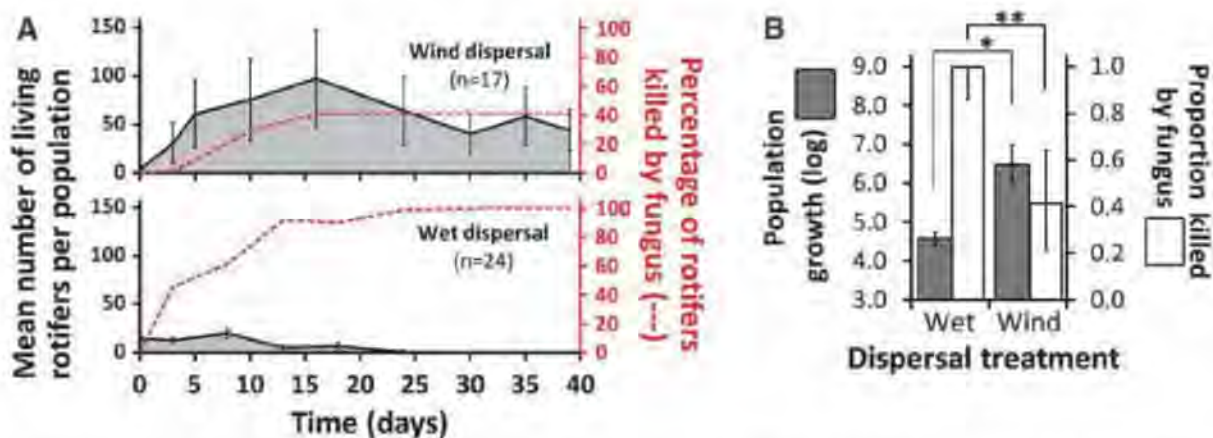
Although facultative or recently evolved asexuality is common among organisms resistant to physical extremes, as well as those with passively dispersed dormant propagules (1), the extraordinary capabilities of the bdelloids in both regards sets them apart. Few animals or plants can withstand complete loss of cellular water, and even then the ability is usually restricted



**Fig. 1.** (A) Growth curve for rotifer populations (*H. elusa*) inoculated with *R. angustispora* versus uninfected controls. Error bars indicate 95% confidence intervals (CIs). (B) Growth curves for inoculated populations subjected to extended desiccation. The proportion of infected rotifers in each dish was used to construct a mean mortality curve. Because all parasitized populations were eventually exterminated, this curve finally stabilizes at a value equal to the proportion of dishes in which infections returned after desiccation. (C) Statistical summary of treatment differences from (B). Population growth was quantified for each dish as the natural logarithm of the total area under its growth curve; shaded bars show the mean for each treatment group ( $\pm 95\%$  CI). Open bars show the proportion of populations that were exterminated by the fungus ( $\pm 95\%$  CI). \* $P < 0.05$  by one-way analysis of variance, Tukey post hoc (for growth), and  $\chi^2$  (for mortality), corrected post hoc.



**Fig. 2. (A)** Growth curves (error bars indicate  $\pm 95\%$  CI) and mortality for 17 *H. elusa* populations founded by artificial wind dispersal of substrate ( $5.01 \text{ mg} \pm 2.52 \text{ SD}$ ) from an infected source culture desiccated for 7 days. Parasites exterminated 7 populations, but 10 remained uninfected (58.8%), compared with complete mortality of all 24 control populations founded concurrently by wet transfer of 5 mg of the same infected source material. **(B)** Statistical summary of differences in (A) [see Fig. 1C legend]; \* $P < 0.0001$ , unpaired  $t$  test; \*\* $P < 0.0001$ , Fisher's exact test.



to specific life stages (e.g., seeds, larvae, eggs) (15). Only bdelloid rotifers and certain tardigrades and nematodes can tolerate repeated bouts of desiccation at any life stage (14, 15), and, of these, only the rotifers occur frequently in samples of rain and wind (16). With their smaller, more aerodynamic tuns, bdelloids can colonize tiny, isolated microhabitats more rapidly than tardigrades and nematodes (17).

The Bdelloidea have been called an “evolutionary scandal” (2), because their ancient asexuality seemed to challenge all hypotheses for the long-term maintenance of sex. However, if anhydrobiotic dispersal enables bdelloid species to escape temporally and spatially from some or many natural enemies, their coevolutionary burden would be substantially reduced. Therefore, our results are consistent with a scenario in which bdelloids have evaded parasites and pathogens over evolutionary time, without incurring the costs of sexuality, by playing a never-ending game of “hide-and-seek” (11, 12). Decoupling from coevolving enemies could help to explain the ancient asexuality of the Bdelloidea under the broad array of models that derive from or incorporate Red Queen dynamics.

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## Objective Confirmation of Subjective Measures of Human Well-Being: Evidence from the U.S.A.

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A huge research literature, across the behavioral and social sciences, uses information on individuals' subjective well-being. These are responses to questions—asked by survey interviewers or medical personnel—such as, “How happy do you feel on a scale from 1 to 4?” Yet there is little scientific evidence that such data are meaningful. This study examines a 2005–2008 Behavioral Risk Factor Surveillance System random sample of 1.3 million U.S. citizens. Life satisfaction in each U.S. state is measured. Across America, people's answers trace out the same pattern of quality of life as previously estimated, from solely nonsubjective data, in one branch of economics (so-called “compensating differentials” neoclassical theory, originally from Adam Smith). There is a state-by-state match ( $r = 0.6$ ,  $P < 0.001$ ) between subjective and objective well-being. This result has some potential to help to unify disciplines.

The concept of human well-being is important but difficult to study empirically. One approach is to listen to what human beings say. Research across the fields of psychology, decision science, medical science, economics, and other social sciences draws upon questionnaire data on people's subjective well-being (1–13). These are numerical scores (e.g., from very satisfied to very dissatisfied) in response to survey

questions such as: How happy are you with your life? Sample sizes in these statistical analyses typically vary from a few dozen individuals in a

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laboratory to many tens of thousands of people in a household survey (2, 4).

If reported well-being numbers provide accurate information about human experience—so are not merely random, deliberately or accidentally untruthful, or irredeemably affected by the possibility that they may not be comparable from one person to another—such data offer important intellectual opportunities to research scientists and practical ones to policy-makers. However, at present, there is little empirical evidence, of the sort able to convince a skeptic, that they do. Perhaps the closest to a validation (of the believability) of subjective well-being scores is the finding that there are correlations between reported happiness and blood pressure, and among emotions, relative reward, and the brain (14–18). This argument, although suggestive, faces a difficulty. The demonstration of a statistical link between physiological measures and subjective well-being answers usefully establishes that the latter are not random numbers. But skeptics can reasonably argue that it does little more than that. Biological indicators are not themselves unambiguous measures of human happiness or unhappiness.

This study focuses not on people but on places (19). Places have characteristics that human beings find objectively pleasant (Hawaiian sunshine or Colorado scenery) and unpleasant (Connecticut land prices or New York City traffic fumes); many are cardinally measurable. The study blends new data from the U.S. Behavioral Risk Factor Surveillance System (BRFSS) (20, 21), elements of the economist's compensating-differentials theory (22–24), and recent research on so-called amenity effects in happiness regression equations (25, 26). The jargon terms “compensating” and “amenity” here capture the following idea. In a country where people can live wherever they please, a place with a harsh environment—think of commuters in Anchorage at 5:30 a.m. in February—has to offer its inhabitants some offsetting feature, often an income advantage, to persuade them to stay there rather than leave to live in a different region. Similarly, intrinsically pleasant environments have to offer less, other things being equal.

The study examines life satisfaction among a recent random sample of 1.3 million U.S. inhabitants. The size of the data set, gathered between 2005 and 2008, provides opportunities denied to previous investigators. The often-used General Social Survey, for example, samples only ~3000 Americans biannually; it is too small to allow state-by-state analysis.

The BRFSS can be downloaded at [www.cdc.gov/BRFSS](http://www.cdc.gov/BRFSS) and is a state-based system of health surveys that gathers (daily) information on health risk behaviors and disease prevalence in America. For many states, it is the only source of up-to-date data. The survey was started in 1984 by the Centers for Disease Control and Prevention (CDC). The data are collected in all 50 states, the District of Columbia, Puerto Rico, the U.S. Virgin Islands, and Guam. The aim is to “identify

emerging health problems, establish and track health objectives, and develop and evaluate public health policies and programs.” Although initially smaller in its sample, 350,000 adults are now interviewed each year; the BRFSS has become the largest (random-digit dialing) telephone health survey in the world. Although the nature of the data set means that it is not possible to record clinical data on people, the advantage is that its samples provide representative snapshots of the self-described health of people in the United States. The supplementary online material (SOM) gives further information (19).

The exact wording of the BRFSS life-satisfaction question is, “In general, how satisfied are you with your life?” Here people are able to answer with one of the following: very satisfied, satisfied, dissatisfied, or very dissatisfied. The questionnaire is publicly available at the BRFSS Web site. Information on BRFSS individuals' life satisfaction was collected for the first time in 2005. Published research on life satisfaction using this data set is thus only beginning.

Life satisfaction in the United States can be treated in a cardinal way by assigning 1 to 4 to the four answers, where “very satisfied” is assigned a 4. The mean of life satisfaction in modern U.S. data are then  $3.4 \pm 0.6$  (SD). Well-being answers are skewed; they are more commonly in the upper end of the possible distribution presented to an interviewee.

Building on now-standard methods used in the literature (4, 9), a life-satisfaction regression equation was estimated, using 1.3 million data points, in which a number of independent variables were included. These were (seven) banded variables for different levels of household income; variables for the survey respondent's age, age squared, and gender; five variables for different ethnic types; and variables for the person's level of education and marital status, as well as for employment status: being self-employed, retired, a student, unemployed, or a homemaker. The sample used in the study was all those respondents between the ages of 18 and 85. Also included as independent control variables were eleven “dummy” variables for the month of interview, and a set of U.S. state dummy variables (because D.C. is included, the regression sample number is 51 states). It can be seen that a dummy variable that is coded zero or unity acts as a  $y$  axis intercept shifter in a regression equation. The study used a linear Ordinary Least Squares estimator in which the four possible satisfaction values of the dependent variable were assigned the integers from a high of 4 to a low of 1, and standard errors were adjusted for clustering at the state level. Substantive findings were not altered by switching to an ordered estimator. The simpler method allows coefficients (given in the first column of Table 1, each of which measures a vertical intercept shifter in a life-satisfaction equation) to be read off as cardinal life-satisfaction points.

The key contribution here—now feasible because of the availability of BRFSS data—is

that it is possible to measure the pattern of people's feelings of well-being (their life-satisfaction scores) across the geography of the United States. It was helpful that the state-by-state dummy-variable pattern of coefficients was not very sensitive to which exact demographic and personal variables were included in the life-satisfaction regression equation.

Information about how much Americans enjoy their lives is shown in Table 1. The numbers in the first column are regression-corrected life-satisfaction patterns across space in the United States; they are state-dummy coefficients. Alabama, because it comes first alphabetically, is the base category against which other states are measured; this normalization makes no difference to the analytical conclusions.

A life-satisfaction regression equation  $L = L(z)$  for individual Americans lies behind Table 1. The statistical structure of these American equations was empirically similar to those found for many industrialized nations within the existing “happiness” literature (19). It controls for (that is, includes as independent  $z$  variables) the incomes and demographic characteristics of sampled individuals. This is necessary to the design of the study's test. The test is not primarily an attempt to assess the different kinds of people who are “happy,” but rather the kinds of geographic areas.

However, there is another, and older, way to try to assess the quality of life across regions. In an elementary form, it continues to be published in magazines in the form of quality-of-life rankings. More formally, it appears in the economics regression-equation literature and uses objective data rather than subjective information. Its focus is people's overall utility (or well-being). The approach relies on the idea that people enjoy having high income and spatial amenities such as sunshine hours, but that they dislike disamenities such as traffic congestion. Regression equations are estimated in which wages, rents, and house prices are the dependent variables. The independent variables in these regression equations are measures of the amenities and disamenities of the areas of the United States. The result is then estimates of “compensating differentials” by geographical area. This allows economists to read off the implied number of dollars (positive or negative) in workers' pay packets in Los Angeles that would be required to offset the unpleasantness of, say, poor air quality, and the pleasantness of high numbers of sunshine hours. The theory of compensating differentials is attributed to the Scottish economist Adam Smith (27).

Some of the most recent and thorough research in this vein, by Stuart Gabriel and colleagues (24), was published in 2003. It used, among other attributes, objective indicators for each U.S. state, such as precipitation, temperature, wind speed, sunshine, coastal land, inland water, public land, National Parks, hazardous waste sites, environmental “greenness,” commuting time, violent crime, air



quality, student-teacher ratio, local taxes, local spending on education and highways, and cost of living. Gabriel's analysis used no subjective data. Instead, these objective data were combined in a weighted average (24), where, as explained in the SOM (19), the weights were not chosen arbitrarily but rather according to coefficient sizes taken from regional wage and price equations.

In nontechnical terms, the compensating-differentials methodology of Gabriel and earlier economists argues—indeed, assumes—that higher house prices are a revealed signal of higher quality of life, other things constant, because humans will move toward the areas they find attractive, which, in turn, drives up housing prices. A spatial equilibrium is reached when a representative citizen obtains the same total utility in each region. This total utility depends on a combination of the inherent quality of life (sunny days, clean air, and so on) plus the earned income from living in that area. This methodology allows these two to be separated conceptually. It is helpful to realize that the Gabriel *et al.* state-by-state quality-of-life ranking is not exceptional. The authors note its approximate similarity to those in earlier writings (23).

The ranking, according to the Gabriel *et al.* methodology, of the inherent quality of life across the U.S. is shown in column 3 of Table 1. The ranking is computed using data for the year 1990,

which appears to be the most recent estimate in the published economics literature. As with column 1, column 3 is not to be thought of as a representation of total utility; instead, it captures nonincome elements of human well-being.

Information on both methods of life-evaluation is provided in Table 1. The first state-dummy coefficient in column 1 of Table 1 can be interpreted as showing that corrected satisfaction-with-life on average in Alaska is 0.013 cardinal life-satisfaction points below that in the base state of Alabama; Arizona is indistinguishable from Alabama, and so on down the listed states. Satisfaction with life is lowest in New York. The particularly high-satisfaction states are Louisiana and Hawaii. In Table 1, the 95% confidence intervals correspond, in each case, to a test of the null hypothesis of zero on the coefficient; it should not be presumed that there is a statistically significant difference between each of the states within low-satisfaction and high-satisfaction groupings of states.

Although it is natural to be guided by formal survey data, it might be thought unusual that Louisiana—a state affected by Hurricane Katrina—comes so high in the state life-satisfaction league table. Various checks were done [discussed in SOM (19)]. It was found that Louisiana showed up strongly before Katrina and in a mental-health ranking done

by Mental Health America and the Office of Applied Studies of the U.S. Substance Abuse and Mental Health Services Administration, based on data from the National Survey on Drug Use and Health 2004–05. Nevertheless, it is likely that Katrina altered the composition of this state—namely, that those who left were not a random sample of the population—so some caution in interpretation is called for about this state's ranked position, and that position may repay future statistical investigation.

Differences in well-being across states are not minor. In cardinal terms, as demonstrated in the SOM (19), they correspond to up to 0.12 life-satisfaction points across U.S. states, which is similar in size to the individual cross-sectional effect on life satisfaction of marital separation or unemployment, other things being equal.

These two approaches are brought together in Fig. 1. It reveals a notable match between the fully adjusted life-satisfaction levels of Table 1 and the objectively calculated Gabriel ranking. The subjective well-being and the objective well-being measures tally. The estimated linear equation is written out in full in Fig. 1 and is approximately of equation form  $y = -0.003 - 0.001x$ .

The gradient in Fig. 1 is negative. This is because the Gabriel method, being a ranking of quality of life across states, uses the convention that 1 is the highest ranking of quality of life

**Table 1.** Two ways of measuring the quality of life in America. The BRFSS life-satisfaction equation uses coefficient dummy-variable values for each state. The compensating-differentials methodology of objective quality-of-life rank, by state, is from Gabriel *et al.* (23). Alabama is included in the data (and in Fig. 1). It

is the base category, against which the other states' coefficients are normalized. In effect, Alabama has a life-satisfaction coefficient of zero [and a ranking of 26 in (23)]. Number of observations, 1,213,992. The standard errors were adjusted for clustering at the state level. CI, confidence interval; N.A., not applicable.

| State                | BRFSS life-satisfaction method |                  |  | Comp diff. methods | State          | BRFSS life-satisfaction method |                  |  | Comp diff. methods |
|----------------------|--------------------------------|------------------|--|--------------------|----------------|--------------------------------|------------------|--|--------------------|
|                      | Coefficient                    | [95% CI]         |  |                    |                | Coefficient                    | [95% CI]         |  |                    |
| Alaska               | -0.013                         | [-0.018, -0.008] |  | 23                 | Montana        | 0.001                          | [-0.002, 0.004]  |  | 4                  |
| Arizona              | 0.001                          | [-0.002, 0.005]  |  | 20                 | Nebraska       | -0.044                         | [-0.047, -0.041] |  | 16                 |
| Arkansas             | -0.017                         | [-0.019, -0.015] |  | 3                  | Nevada         | -0.065                         | [-0.068, -0.062] |  | 29                 |
| California           | -0.076                         | [-0.080, -0.072] |  | 42                 | New Hampshire  | -0.033                         | [-0.036, -0.030] |  | 43                 |
| Colorado             | -0.027                         | [-0.030, -0.024] |  | 34                 | New Jersey     | -0.078                         | [-0.081, -0.075] |  | 47                 |
| Connecticut          | -0.081                         | [-0.084, -0.078] |  | 32                 | New Mexico     | -0.029                         | [-0.034, -0.024] |  | 14                 |
| Delaware             | -0.027                         | [-0.029, -0.025] |  | 30                 | New York       | -0.088                         | [-0.090, -0.085] |  | 50                 |
| District of Columbia | -0.048                         | [-0.051, -0.045] |  | N.A.               | North Carolina | -0.013                         | [-0.015, -0.012] |  | 17                 |
| Florida              | 0.004                          | [0.002, 0.006]   |  | 10                 | North Dakota   | -0.030                         | [-0.032, -0.027] |  | 6                  |
| Georgia              | -0.021                         | [-0.023, -0.020] |  | 36                 | Ohio           | -0.070                         | [-0.071, -0.068] |  | 33                 |
| Hawaii               | 0.011                          | [0.004, 0.018]   |  | 38                 | Oklahoma       | -0.026                         | [-0.029, -0.024] |  | 21                 |
| Idaho                | -0.014                         | [-0.017, -0.011] |  | 5                  | Oregon         | -0.040                         | [-0.044, -0.037] |  | 22                 |
| Illinois             | -0.072                         | [-0.074, -0.069] |  | 48                 | Pennsylvania   | -0.067                         | [-0.069, -0.065] |  | 35                 |
| Indiana              | -0.078                         | [-0.080, -0.077] |  | 44                 | Rhode Island   | -0.068                         | [-0.071, -0.066] |  | 12                 |
| Iowa                 | -0.041                         | [-0.044, -0.038] |  | 15                 | South Carolina | 0.001                          | [0.000, 0.002]   |  | 18                 |
| Kansas               | -0.044                         | [-0.046, -0.041] |  | 19                 | South Dakota   | -0.014                         | [-0.017, -0.010] |  | 2                  |
| Kentucky             | -0.045                         | [-0.047, -0.043] |  | 24                 | Tennessee      | 0.003                          | [0.001, 0.004]   |  | 28                 |
| Louisiana            | 0.033                          | [0.032, 0.034]   |  | 8                  | Texas          | -0.014                         | [-0.018, -0.010] |  | 25                 |
| Maine                | -0.006                         | [-0.009, -0.003] |  | 9                  | Utah           | -0.026                         | [-0.030, -0.023] |  | 39                 |
| Maryland             | -0.066                         | [-0.069, -0.064] |  | 45                 | Vermont        | -0.017                         | [-0.020, -0.014] |  | 13                 |
| Massachusetts        | -0.070                         | [-0.073, -0.068] |  | 27                 | Virginia       | -0.033                         | [-0.035, -0.031] |  | 31                 |
| Michigan             | -0.079                         | [-0.081, -0.077] |  | 49                 | Washington     | -0.046                         | [-0.049, -0.042] |  | 41                 |
| Minnesota            | -0.031                         | [-0.034, -0.027] |  | 46                 | West Virginia  | -0.044                         | [-0.046, -0.042] |  | 11                 |
| Mississippi          | 0.001                          | [0.000, 0.001]   |  | 7                  | Wisconsin      | -0.038                         | [-0.040, -0.035] |  | 37                 |
| Missouri             | -0.064                         | [-0.066, -0.062] |  | 40                 | Wyoming        | -0.013                         | [-0.016, -0.010] |  | 1                  |



(and hence the number 50, for the state of New York, corresponds to the lowest quality-of-life area in the United States). The calculated Pearson  $r$  coefficient is 0.6. With 48 degrees of freedom, the null hypothesis of no correlation is therefore rejected at the  $P < 0.001$  level (in fact, approximately  $P = 0.0001$ ) on a two-tailed test. The  $r^2$  is 0.36; that is, ~36% of the variance in the  $y$  axis variable is explained by the  $x$  axis variable. As shown more fully in the SOM (19) and in (20), the high-satisfaction states in Fig. 1 are not simply the high-income ones.

A correlation coefficient of 0.6 is unusual by the standards of behavioral science. It is high by the cut-offs suggested by Cohen's (28) rules-of-thumb (which argued that in human data an  $r$  value over 0.5 should be seen as a large association, and 0.3 a medium one). An  $r = 0.6$  is the same degree of correlation, for example, as has been found for people's own life-satisfaction readings taken 2 weeks apart (that is, using the same well-being question, asked of the same person) (29). An  $r = 0.3$  has been demonstrated for subjective well-being correlated with EEG asymmetry in the brain (15). The variable on the  $x$  axis in Fig. 1 is an ordinal rank. As would be expected, an arguably preferable Spearman rank test confirms the same relation as the Pearson test. An alternative estimation method would be multilevel modeling (30, 31). The data set is too large to allow this easily, but experiments on subsamples were done. These suggested that equivalent life-satisfaction equations emerge.

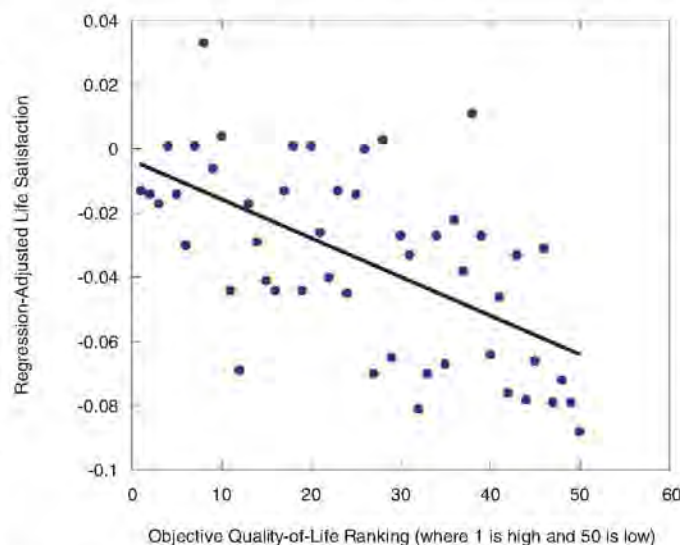
Although this study is designed as an analytical contribution, measures of subjective well-

being are becoming more widely discussed outside the laboratory. As the draft of this article was being revised, the Stiglitz-Sen-Fitoussi Commission on the Measurement of Social and Economic Progress (32), set up by Nicholas Sarkozy to assess the appropriate goals of Western governments over the remainder of the century, issued a report. Worldwide attention was garnered for its argument: "A ... unifying theme of the report ... is that the time is ripe for our measurement system to shift emphasis from measuring economic production to measuring people's well-being." "Measures of both objective and subjective well-being provide key information about people's quality of life. Statistical offices [worldwide] should incorporate questions to capture people's life evaluations, hedonic experiences and priorities in their own survey." [Executive Summary of Commission Report (32), pp. 12 and 16].

In this vein, the current study's principal contribution, Fig. 1, offers a possible bridge between different ways of thinking—between, in particular, the fields of hedonic psychology and neoclassical economics (the latter has traditionally been hostile to the use of data on subjectively reported feelings). It offers a cross-check on the spatial compensating-differentials theory of economics and regional science. It may also be relevant to the work of behavioral scientists, geographers, applied psychologists, and mental-health specialists. The study's finding suggests that subjective well-being data contain genuine information about the quality of human lives.

**Fig. 1.** Fitted equation: Adjusted Life Satisfaction =  $-0.0035 - 0.0012$  Objective Rank;  $r = 0.598$ . Each dot is a state. The correlation is significant at  $P < 0.001$  on a two-tailed test. This figure plots state dummy coefficients from a life-satisfaction equation against state rank in quality of life from the compensating-differentials results, based on objective amenities like sunshine hours, of Gabriel *et al.* (24). Life satisfaction is coded for each individual from a score of 4 (very satisfied) to 1 (very dissatisfied). On the  $y$  axis, the regression controls for household income, as well as the survey respondent's gender, age, age squared, education, marital status, employment status, and race, and also year dummies and month-of-interview dummies. Alabama is included. Washington, DC, is omitted from (24) and thus from here. The bottom right-hand observation is New York. Wording of the question in the BRFSS questionnaire (questionnaire line 206):

In general, how satisfied are you with your life?  
 1 Very satisfied  
 2 Satisfied  
 3 Dissatisfied  
 4 Very dissatisfied



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33. For discussions and detailed points, we thank four referees and P. J. Hammond, M. Kimball, D. Krupka, E. Luttmer, J. Miles, E. Proto, and D. Sgroi. Valuable suggestions were made by D. Benjamin, D. Blanchflower, A. Clark, M. Clements, E. Diener, D. Dorling, D. Gilbert, A. Goodall, C. Graham, O. Heffetz, S. Luechinger, S. Mukherjee, R. Putnam, and J. Smith. The first author's work was supported by a U.K. Economic and Social Research research professorship. No conflicts of interest.

## Supporting Online Material

[www.sciencemag.org/cgi/content/full/science.1180606/DC1](http://www.sciencemag.org/cgi/content/full/science.1180606/DC1)  
 Materials and Methods  
 Figs. S1 and S2  
 Tables S1 to S4  
 References

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# Platelets Amplify Inflammation in Arthritis via Collagen-Dependent Microparticle Production

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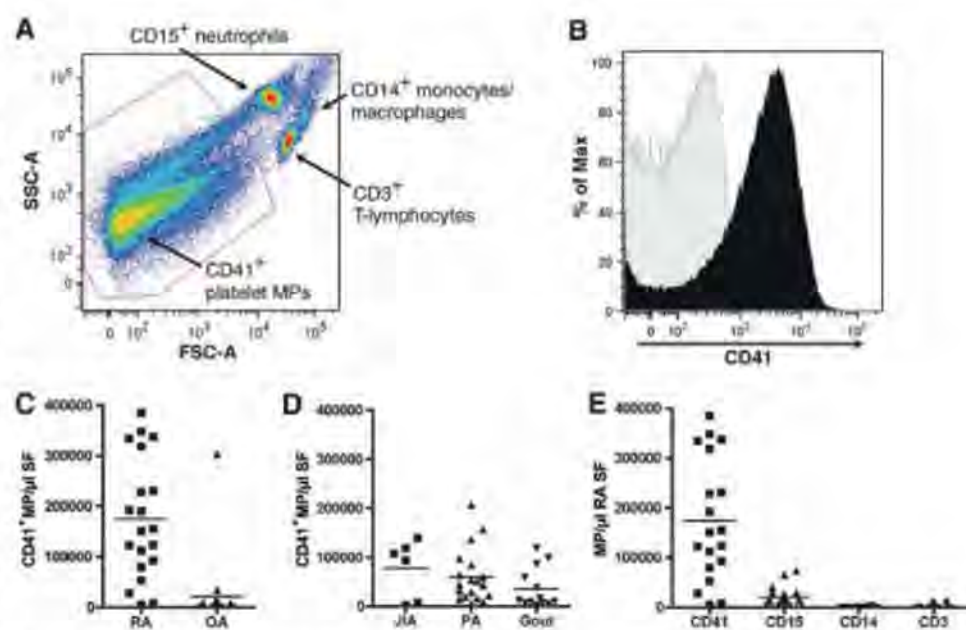
In addition to their pivotal role in thrombosis and wound repair, platelets participate in inflammatory responses. We investigated the role of platelets in the autoimmune disease rheumatoid arthritis. We identified platelet microparticles—submicrometer vesicles elaborated by activated platelets—in joint fluid from patients with rheumatoid arthritis and other forms of inflammatory arthritis, but not in joint fluid from patients with osteoarthritis. Platelet microparticles were proinflammatory, eliciting cytokine responses from synovial fibroblasts via interleukin-1. Consistent with these findings, depletion of platelets attenuated murine inflammatory arthritis. Using both pharmacologic and genetic approaches, we identified the collagen receptor glycoprotein VI as a key trigger for platelet microparticle generation in arthritis pathophysiology. Thus, these findings demonstrate a previously unappreciated role for platelets and their activation-induced microparticles in inflammatory joint diseases.

Platelets are highly abundant hematopoietic cells, outnumbering leukocytes in the peripheral circulation by almost two orders of magnitude (1). The role of platelets in hemostasis and wound repair after vascular injury is well known (2); however, there is a growing appreciation for their role in inflammation. This role has been studied most carefully in atherosclerosis, a chronic inflammatory disease of the blood vessels in which platelets release a broad range of inflammatory mediators that support endothelial cell activation, leukocyte adhesion and transmigration, monocyte maturation, and elaboration of cytokines and reactive oxygen species [reviewed in (3)].

The growing literature regarding the proinflammatory capacity of platelets prompted us to investigate whether they could participate in another common inflammatory condition, inflammatory arthritis. Of the inflammatory arthritides, rheumatoid arthritis (RA) is the most common (4). RA manifests as chronic inflammation of the synovial lining of the joint, resulting in pain, swelling, and ultimately destruction of cartilage and bone (5). Although evidence has implicated lymphocytes, innate immune cells such as neutrophils and mast cells, and synovial tissue cells in the evolution of RA, to date platelets have no known functional role. We first sought to determine whether platelets are present in

RA synovial fluids (SF) by use of the platelet-specific marker CD41 (GPIIb/α2b from the platelet-specific integrin GPIIb/IIIa/α2bβ3) (6, 7). Flow cytometric analyses detected a substantial number of CD41-positive events in RA SF (Fig.

1, A and B). Unexpectedly, most CD41-positive events were smaller in size than intact leukocytes or platelets (fig. S1), suggesting that these particles are platelet microparticles (MPs). Platelet MPs are intact vesicles (0.2 to 1 μm in diameter) that form by budding from the membranes of activated platelets (8–10). We found on average just under  $2 \times 10^5$  CD41<sup>+</sup> MPs per microliter of SF from patients with RA (Fig. 1C). In contrast to RA SF, where MPs were present in all samples, CD41<sup>+</sup> MPs were undetectable in 19 out of 20 osteoarthritis (OA) SF samples (Fig. 1C). We also detected platelet MPs in other inflammatory arthritides (Fig. 1D). We further investigated the presence of MPs originating from other hematopoietically derived cell types. Consistent with previous observations (11), MPs expressing neutrophil, T cell, or macrophage markers were present albeit in substantially smaller amounts than observed for platelet MPs in RA SF (Fig. 1E). Recognizing that platelets can adhere to migrating leukocytes (12, 13), we also assessed the presence of platelets associated with SF leukocytes. Intriguingly, we found that a substantial number of SF leukocytes costained with CD41 and the leukocyte marker CD45 (fig. S1, D and E). Microscopic examination revealed that CD41 staining in these cells was restricted to discrete particles of MP size adherent to the leukocyte surface; we could not detect



**Fig. 1.** Platelet MPs are abundant in inflammatory SF. Cells in freshly isolated RA SF were stained with lineage markers: CD15 (neutrophils), CD3 (T cells), CD14 (monocytes and macrophages) and CD41 (platelets), or the appropriate isotype controls and analyzed by flow cytometry. (A) Forward- by side-scatter profiles of events in RA SF. Populations identified by further gating and lineage marker staining are labeled. (B) Representative histogram of CD41<sup>+</sup> (black fill) platelet MPs resident in RA SF. Events were gated based on the forward-scatter parameters indicated in (A). (Gray fill, isotype control.) Data are representative of profiles from eight RA patients. (C) Flow cytometric quantification of CD41<sup>+</sup> platelet MPs (<1 μm as determined by size calibration beads) in RA and OA SF after removal of leukocytes by centrifugation ( $n = 20$  donors per group). (D) Flow cytometric quantification of platelet (CD41<sup>+</sup>) MPs in SF from juvenile idiopathic arthritis (JIA,  $n = 6$ ), psoriatic arthritis (PA,  $n = 19$ ), and gout ( $n = 14$ ). (E) Flow cytometric quantification of MPs in RA SF derived from the indicated cell types ( $n = 19$  donors).

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intact platelets associated with SF leukocytes (fig. S1D).

We next explored the pathophysiologic importance of platelets and platelet MPs in inflammatory arthritis in vivo. Here, we used the K/BxN serum transfer model of inflammatory arthritis. The progressive distal symmetric erosive polyarthritis observed in K/BxN T cell receptor transgenic mice results from T cell recognition of a ubiquitous autoantigen, glucose-6-phosphate isomerase, presented by major histocompatibility complex class II I-A<sup>b</sup>, driving high-titer arthritogenic autoantibody production [reviewed in (14)]. Arthritis can also be induced by passive transfer of immunoglobulin G (IgG) autoantibodies from K/BxN mice into wild-type mice. Numerous effector mechanisms have been implicated in the pathogenesis of the IgG-driven effector phase of K/BxN serum-transfer arthritis, including neutrophils, mast cells, Fc gamma receptors, and soluble mediators [interleukin-1 (IL-1), tumor necrosis factor (TNF), complement C5a/C5a receptor, eicosanoids, and the mast cell protease tryptase] (14–16). To study the role of platelets in this system, we initiated K/BxN serum transfer arthritis in animals treated with a platelet-depleting antibody regimen that rapidly (within 60 min) reduces platelet numbers by >95% for at least 6 days (17). We found that platelet-depleted mice exhibited a marked reduction in arthritis as assessed by clinical scoring and by histological analysis (Fig. 2, A and B).

These findings demonstrate that platelets are required for inflammatory arthritis development in vivo.

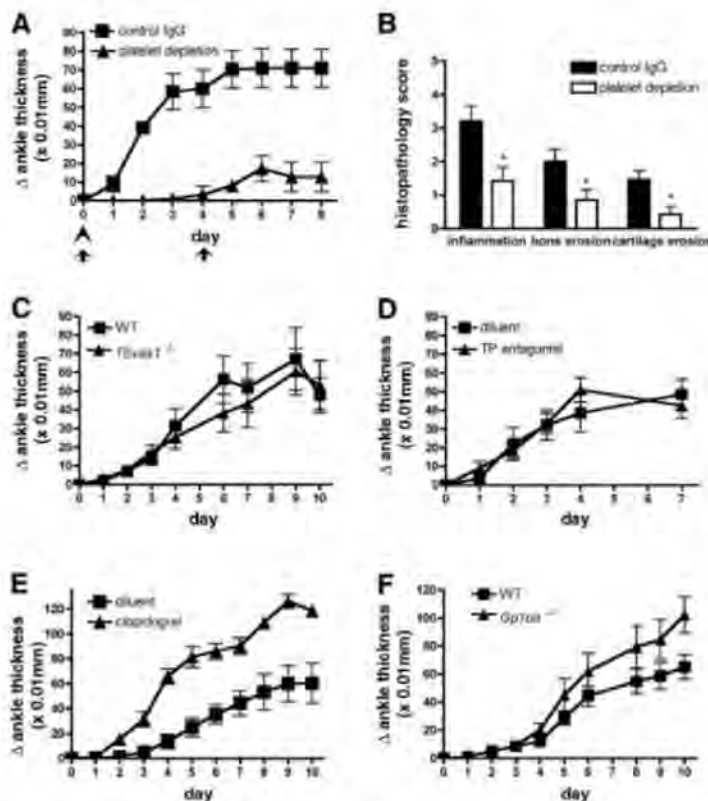
To gain further insight into the link between platelets and joint inflammation, we explored the mechanisms by which platelets are activated to release MP in the context of arthritis. Platelets can be triggered via several pathways, many of which have already been targeted for the prevention of thrombosis. Among these pathways we considered were thromboxane A2 stimulation of its receptor (TP) on platelets (blocked by TP antagonist SQ 29548), ligation of the P2Y12 receptor by adenosine 5'-diphosphate (inhibited by clopidogrel), and GPIb-IX, a platelet membrane glycoprotein complex that binds to von Willebrand factor. By using genetically deficient mice and pharmacologic blockade, we determined that interference with these pathways did not impede development of joint inflammation, suggesting that these pathways do not regulate platelet MP generation in inflammatory arthritis (Fig. 2, C to G).

What other pathways to platelet activation could be operative in arthritis? Knowing that the concentration of platelet-derived MPs within RA SF ( $2 \times 10^5$  per microliter) greatly exceeds that in RA peripheral blood (600 per microliter) (18), we hypothesized that platelet activation likely occurs locally. The vasculature of the joint is in intimate contact with fibroblast-like synoviocytes (FLS) and the extracellular matrix

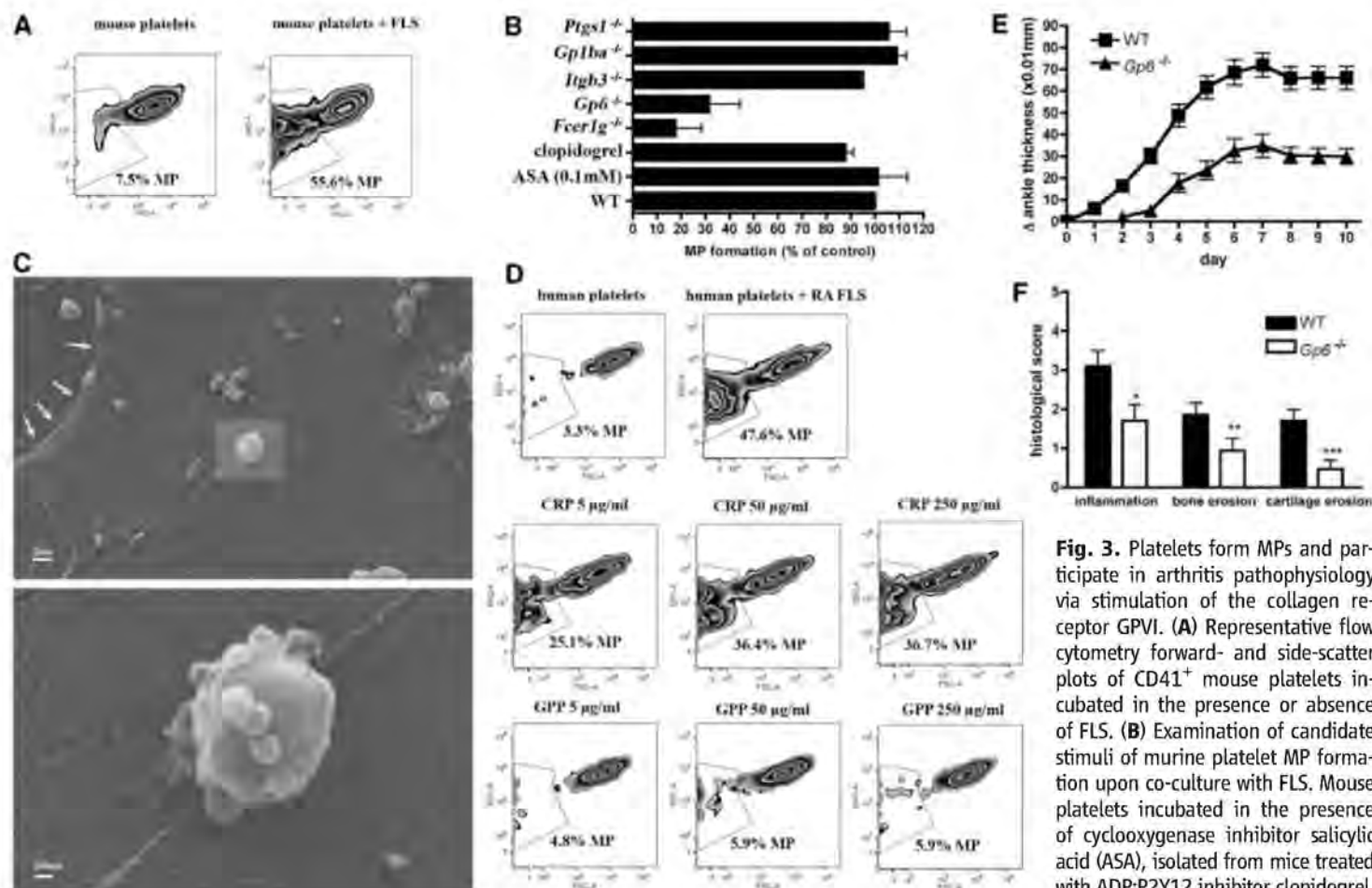
(ECM) elaborated by these cells (19), and thus we modeled the interaction of platelets with synoviocytes and their ECM in vitro. Mouse platelets coincubated with primary mouse FLS promptly released MPs (Fig. 3A). To determine the trigger for MP release, we assessed MP formation after treatment with specific pharmacologic inhibitors or using platelets isolated from mice deficient in candidate genes. Consistent with our in vivo results, we found that cyclooxygenase (*Ptgs1*<sup>-/-</sup>), thromboxane, GPIIb/IIIa (*Itgb3*<sup>-/-</sup>), GPIb (*Gp1ba*<sup>-/-</sup>), and ADP-P2Y12 pathways were dispensable (Fig. 3B). We next explored whether the ECM generated by primary cultured FLS could be the relevant stimulus, particularly collagen. Previous studies have demonstrated that collagen can activate platelets to form MPs (8) and that glycoprotein VI (GPVI) is the predominant collagen receptor on platelets (20). GPVI is an immunoglobulin superfamily member expressed exclusively by megakaryocytes and platelets that signals via noncovalent interaction with the common  $\gamma$ -chain of the Fc receptor (21). Using platelets lacking either FcR- $\gamma$ -chain (*Fcgr1g*<sup>-/-</sup>) or GPVI (*Gp6*<sup>-/-</sup>), we found that generation of MPs by primary FLS was mediated predominantly via this pathway (Fig. 3B). After confirming that human platelets also released MPs upon coincubation with primary human FLS (Fig. 3, C and D), we validated the relevance of GPVI in MP release in humans using the GPVI-specific agonist collagen-related peptide (CRP) (22) (Fig. 3D). Further characterization of collagen-stimulated platelet MPs showed that their phenotype is congruent with that of platelet MPs from SF and distinct from that of intact platelets (table S1). Finally, we assessed whether GPVI is relevant for platelet activation in vivo by administering K/BxN serum to *Gp6*<sup>-/-</sup> and control mice and assessing the development of synovitis. Both clinical and histomorphometric assessment confirmed that arthritis in *Gp6*<sup>-/-</sup> mice was significantly reduced (Fig. 3, E and F). These results confirm that activation of platelets via the collagen receptor GPVI—a pathway resulting in MP generation—plays an important role in the pathogenesis of arthritis.

Having demonstrated platelet participation in inflammatory arthritis in vivo, we aimed to identify platelet MP effector activities that contribute to joint inflammation. The most abundant cell in the pathologic rheumatoid pannus tissue is the FLS (19). This lineage plays a substantial role in the perpetuation of joint inflammation and in the destruction of cartilage (19, 23). We surveyed the capacity of collagen-stimulated human platelet MPs to elicit a range of cytokines and chemokines from FLS and observed prominent production of the broadly inflammatory cytokine IL-6 and the neutrophil chemoattractant IL-8 (Fig. 4A and fig. S2). Consistent with this observation, incubation of MPs isolated from RA SF induced FLS to release substantial quan-

**Fig. 2.** Platelets are involved in arthritis development. (A) Arthritis severity after K/BxN serum transfer in mice administered a platelet-depleting antibody (triangles) or isotype control (squares);  $n = 10$  mice per group. Data are the mean  $\pm$  SEM pooled from two independent experiments.  $P < 0.001$ . Arrows, parenteral administration of platelet-depleting antibody; arrowheads, K/BxN serum administration. (B) Histomorphometric quantification of arthritis severity in ankle joints of platelet-depleted and control mice at experiment termination;  $n = 10$  mice per group.  $*P < 0.01$ . (C to F) Arthritis severity was measured after administration of K/BxN serum in mice (C) deficient in thromboxane synthase (*Tbxas1*<sup>-/-</sup>), treated daily with (D) the thromboxane A2 (TP) antagonist SQ 29,548 or (E) ADP:P2Y12 inhibitor clopidogrel, or in mice deficient in (F) GPIb (*Gp1ba*<sup>-/-</sup>). Data are the mean  $\pm$  SEM,  $n = 10$  mice per group. (C), (D), (F)  $P$  = not significant; (E)  $P < 0.001$ .







**Fig. 3.** Platelets form MPs and participate in arthritis pathophysiology via stimulation of the collagen receptor GPVI. **(A)** Representative flow cytometry forward- and side-scatter plots of CD41<sup>+</sup> mouse platelets incubated in the presence or absence of FLS. **(B)** Examination of candidate stimuli of murine platelet MP formation upon co-culture with FLS. Mouse platelets incubated in the presence of cyclooxygenase inhibitor salicylic acid (ASA), isolated from mice treated with ADP:P2Y12 inhibitor clopidogrel, or from the indicated gene-targeted

mice were coincubated with mouse FLS. MP formation was quantified by flow cytometry. Data are the mean  $\pm$  SEM,  $n = 3$  independent experiments in duplicate. **(C)** Scanning electron micrograph of human platelets exhibiting MP budding when incubated in the presence of FLS. Arrows indicate the edge of the fibroblast-like synoviocyte. Upper and lower panels are 9800 $\times$  and 69,270 $\times$  magnifications, respectively. **(D)** Human platelets form MPs when incubated with FLS and when exposed to a GPVI-specific peptide ligand (CRP) but not in the presence of a related control peptide (GPP). **(E)** Arthritis severity after K/BxN serum transfer was quantified in GPVI-null (*Gp6*<sup>-/-</sup>) (triangles) or wild-type control (squares) mice. Data are the mean  $\pm$  SEM pooled from three independent experiments;  $n = 25$  mice per group.  $P < 0.001$ . **(F)** Histomorphometric quantification of arthritis severity in ankle tissues from GPVI-null (*Gp6*<sup>-/-</sup>) and WT mice at experimental termination. Data are the mean  $\pm$  SEM;  $n = 25$  mice per group. \* $P = 0.019$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .

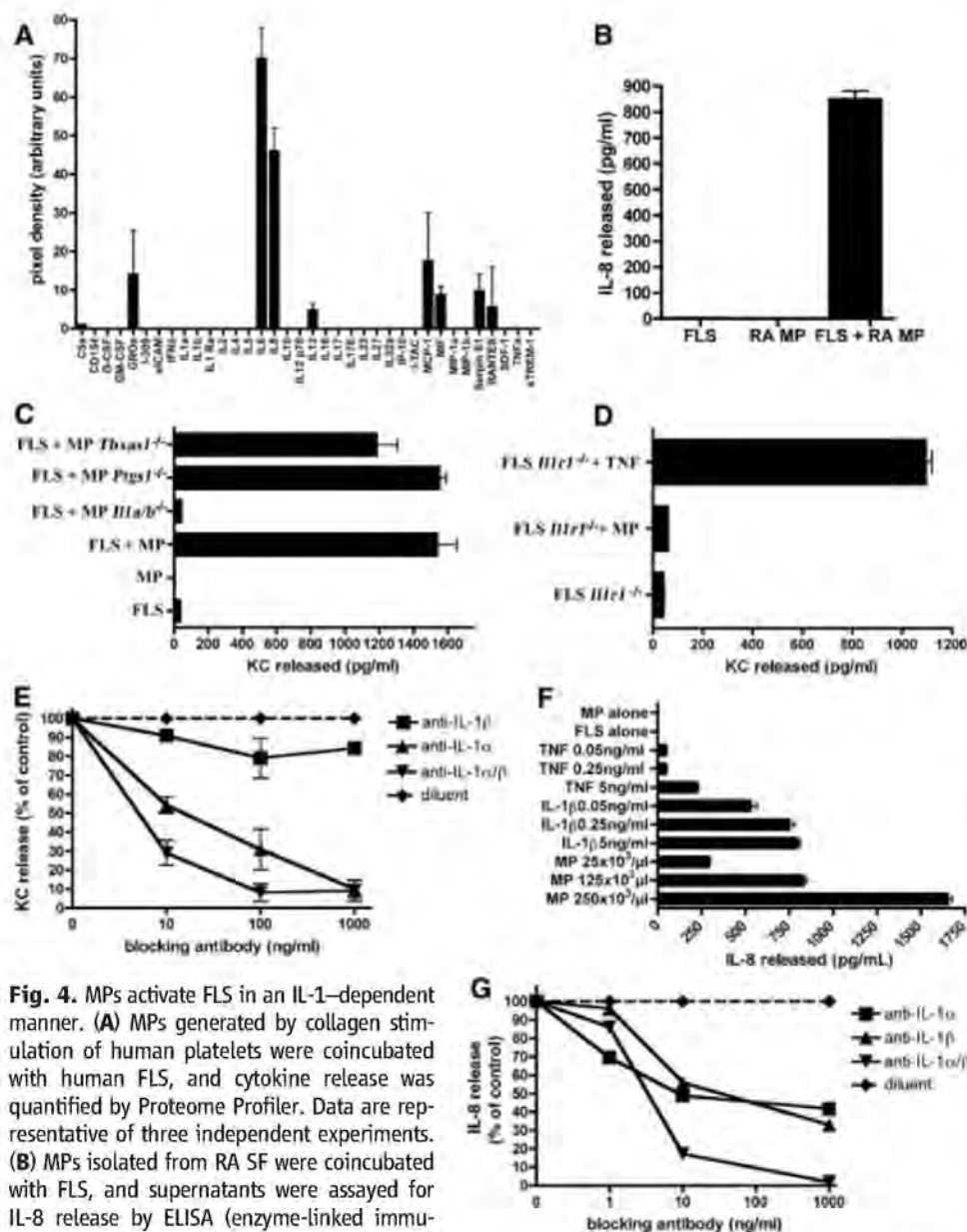
ties of both cytokines (Fig. 4B and fig. S3). We focused our further studies on MP stimulation of IL-8 by FLS because SF from the inflammatory arthritides is rich in neutrophils. To elucidate a mechanism by which platelet MPs stimulate FLS, we used a genetic approach with MPs generated from mice deficient in specific candidate genes. We found that platelet MPs generated from mice lacking both IL-1 $\alpha$  and IL-1 $\beta$  (*Il1a/b*<sup>-/-</sup>) were incapable of stimulating murine FLS to produce the murine IL-8 ortholog KC (Fig. 4C). Furthermore, FLS generated from mice deficient in the IL-1 receptor (*Il1r1*<sup>-/-</sup>) were unresponsive to platelet MPs, though release of KC remained intact after TNF stimulation (Fig. 4D). By contrast, MPs from mouse platelets deficient in prostaglandin synthesis capacity (*Ptgs1*<sup>-/-</sup>) retained their ability to stimulate KC production from FLS (Fig. 4C).

Platelets exhibit membrane-associated IL-1 activity (24), and we confirmed that both forms of this cytokine were present in wild-type murine

MPs, although IL-1 $\alpha$  was predominant (IL-1 $\alpha$ ,  $87 \pm 7$  pg/mg protein; IL-1 $\beta$ ,  $2 \pm 0.2$  pg/mg protein). Blocking both forms of IL-1 with neutralizing antibodies was necessary to fully blunt FLS activation by MPs (Fig. 4E). We obtained similar results in the human system. Platelet MPs from RA SF expressed surface IL-1 $\alpha$ , which as in murine MPs predominated over IL-1 $\beta$  (fig. S4). Similarly, human platelet MPs elicited by in vitro collagen stimulation expressed both IL-1 $\alpha$  and IL-1 $\beta$  (19.1 versus 0.1 pg/mg protein) and triggered RA FLS to release IL-8 in a dose-dependent manner, indeed, more robustly than either IL-1 $\beta$  or TNF (Fig. 4F). Both forms of IL-1 participated in human FLS stimulation because neutralization of platelet MP IL-1 activity required blocking antibodies against both IL-1 $\alpha$  and IL-1 $\beta$  (Fig. 4G). Together, these results show that platelet MPs likely contribute to joint inflammation via mechanisms including highly potent IL-1-mediated activation of resident synoviocytes.

These results provide an explanation for several disparate previous observations. Radio-labeled platelets localize to inflamed joints (25) and RA SF displays appreciable amounts of soluble platelet proteins (26), yet intact platelets are rare in arthritic SF. Whereas SF MP concentrations exceed those in the circulation of RA patients by several orders of magnitude (18), platelet activation appears to be primarily an articular process, wherein MPs disseminate platelet-derived cytokines into the arthritic joint. Subsynovial capillaries exhibit fenestrations and are prone to enhanced permeability after stimulation (27, 28). We speculate that circulating platelets contact ECM via these fenestrations when local conditions favor permeability, activating GPVI. Alternatively, platelet "sampling" of ECM via fenestrations may be routine, activating platelets as ECM constituents are modified. Thus, synovium is enriched for collagen type IV (29), and we have observed that FLS deficient in collagen type IV demonstrate





**Fig. 4.** MPs activate FLS in an IL-1-dependent manner. (A) MPs generated by collagen stimulation of human platelets were coincubated with human FLS, and cytokine release was quantified by Proteome Profiler. Data are representative of three independent experiments. (B) MPs isolated from RA SF were coincubated with FLS, and supernatants were assayed for IL-8 release by ELISA (enzyme-linked immunosorbent assay). (C) Mouse platelet MPs generated by collagen stimulation of platelets from the indicated genotypes were coincubated with mouse FLS, and supernatants were assayed for KC release by ELISA. (D) Mouse MPs generated by collagen stimulation of WT platelets were coincubated with IL-1R1-null (*Il1r1*<sup>-/-</sup>) FLS, and supernatants were assayed for KC release by ELISA. Recombinant TNF (10 ng/ml) was added to FLS to induce KC release as a positive control. (E) Mouse platelet MPs were coincubated with FLS in the presence of IL-1-neutralizing antibodies, and supernatants were assayed for KC release by ELISA. (F) Potency of human MP stimulation of FLS. FLS were exposed to graded concentrations of IL-1 $\beta$ , TNF, or platelet MPs, and IL-8 release was quantified in culture supernatants by ELISA. (G) Human platelet MPs were coincubated with FLS in the presence of IL-1-neutralizing antibodies, and supernatants were assayed for IL-8 release by ELISA. Data for (B) to (G) are the mean  $\pm$  SEM of three independent experiments.

reduced capacity to stimulate platelet MP release (fig. S5).

Other pathways linking platelets to arthritis await discovery. The effect of GPVI deficiency on generating MPs and suppressing arthritis is incomplete, and IL-1 is unlikely to be the only relevant platelet mediator in the synovial environment. Further, we have observed direct binding of MPs by SF leukocytes (fig. S1, D and E), an interaction whose functional consequences remain unknown.

The relevance of these results for human disease is substantial. Because mice or humans lacking GPVI remain healthy (30), antagonism of this receptor represents a novel therapeutic approach. The observation that membrane-associated MP IL-1 is unusually difficult to antagonize (Fig. 4) may help to explain the limited effect of IL-1 blockade in RA (31), and the prominence of IL-1 $\alpha$  within MPs poses potential constraints on the efficacy of IL-1 $\beta$ -specific

agents. Our results provide compelling evidence that platelets play an amplifying role in the pathophysiology of inflammatory arthritis, liberating proinflammatory MPs that represent the most abundant cellular element in SF.

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#### Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5965/580/DC1  
Materials and Methods  
Figs. S1 to S5  
Table S1  
References

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# Decorrelated Neuronal Firing in Cortical Microcircuits

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Correlated trial-to-trial variability in the activity of cortical neurons is thought to reflect the functional connectivity of the circuit. Many cortical areas are organized into functional columns, in which neurons are believed to be densely connected and to share common input. Numerous studies report a high degree of correlated variability between nearby cells. We developed chronically implanted multitetrode arrays offering unprecedented recording quality to reexamine this question in the primary visual cortex of awake macaques. We found that even nearby neurons with similar orientation tuning show virtually no correlated variability. Our findings suggest a refinement of current models of cortical microcircuit architecture and function: Either adjacent neurons share only a few percent of their inputs or, alternatively, their activity is actively decorrelated.

Correlated response fluctuations among simultaneously recorded neurons have been observed in a number of cortical areas (1–14). The prevailing hypothesis is that these correlations (referred to as “noise correlations”) are caused by random fluctuations in the activity of neurons presynaptic to a pair of cells (5, 6, 15–17). Noise correlations are reported to be particularly strong in nearby cells with similar response properties (5–13, 18), which supports the idea that nearby cells within a functional column are densely connected and share a substantial amount of common input (17). Theoretical work shows that noise correlations with such a “limited-range” structure are particularly detrimental for population coding (5, 19–21). Thus, knowledge of the precise nature of noise correlations can advance our understanding of the structure and function of cortical microcircuits in vivo.

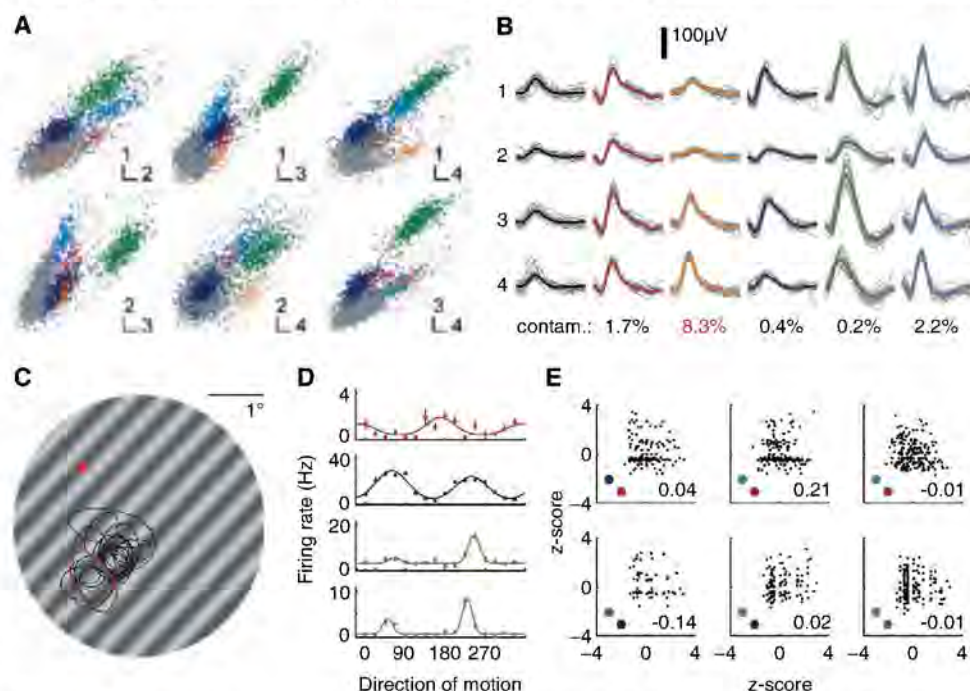
Although the prevalent finding of spike count correlations in the range of 0.1 to 0.3 (2–11) seems to suggest that their magnitude and cause have been firmly established, there are several technical challenges in the measurement of noise correlations. Spike count correlations can be generated in the absence of shared presynaptic noise by a number of factors: First, it is difficult to control for internal variables, which modulate firing rates, such as motor plans, or cognitive states, like attention. Second, recordings from electrodes that are not chronically implanted often suffer from instabilities in the electrodes’ positions. Third, if multiple cells are recorded from the same electrode, suboptimal single-unit isolation is a concern (22). Fourth, in experiments conducted under anesthesia, correlations may arise from spon-

taneous oscillations that are absent in behaving animals (23). Given that all these factors will artificially increase estimates of noise correlations, it is important to control for every single one.

We reexamined this issue and measured spike count correlations by using arrays of chronically implanted tetrodes (fig. S1) to simultaneously record the activity of local groups of neurons in the primary visual cortex (area V1) of awake monkeys (24, 25). Tetrodes provide a superior quality of single-unit isolation of nearby neurons (26) compared with conventional single electrodes or rigid multielectrode arrays. An example of a tetrode isolating multiple cells is shown in Fig. 1. Because

the signal is recorded simultaneously by four adjacent microwires, the location of the neurons can be triangulated, resulting in distinct clusters, each representing the action potentials of a single neuron (Fig. 1, A and B). If, for example, only channel 4 had been recorded (as could be the case with a single electrode), cells 1, 2, 4, and 5 would have been nearly impossible to distinguish. For our analyses, we only considered cells that were quantitatively determined to be very well isolated, in this case discarding the second neuron, which had ~8% falsely assigned spikes (25). Cells recorded on one tetrode had highly overlapping receptive fields (Fig. 1C, colored outlines). We presented sine wave gratings drifting in 16 directions of motion perpendicular to the grating orientation (Fig. 1C). Consistent with the columnar organization of V1, three of the four neurons had very similar preferred orientations (Fig. 1D). When we examined the spike count correlations ( $r_{sc}$ ) of the six pairs, they were extremely low (Fig. 1E), with an  $r_{sc}$  average value of 0.02.

We collected data in a total of 46 recording sessions from two monkeys (D, 27; H, 19). Gratings were presented at eight different orientations and were either static or drifting in the direction orthogonal to the orientation. The gratings were large enough to cover the receptive fields of all neurons recorded by the array (Fig. 1C). Spatial frequency and speed were chosen such that a large number of neurons was driven,



**Fig. 1.** Example of five single units recorded from one tetrode. Colors are matched in all panels. (A) Scatter plots showing amplitude of first principal component of spike waveforms for all pairs of channels. Clusters are modeled as multivariate Gaussians, which allows quantification of their separation. (B) Example waveforms of multiunit (black) and the five single units (colored). Each row corresponds to one tetrode channel. Estimated false-assignment rates (25) are shown below each column. Neuron 2 (orange) is discarded because of insufficient isolation. (C) Grating stimulus overlaid with receptive field outlines of 24 simultaneously recorded neurons. Red dot, fixation spot. (D) Tuning curves. Error bars are SEM. (E) Scatter plots of z-score-transformed responses for all pairs obtained from the four neurons.  $r_{sc}$  values are indicated. Pair identities are coded by colored dots.

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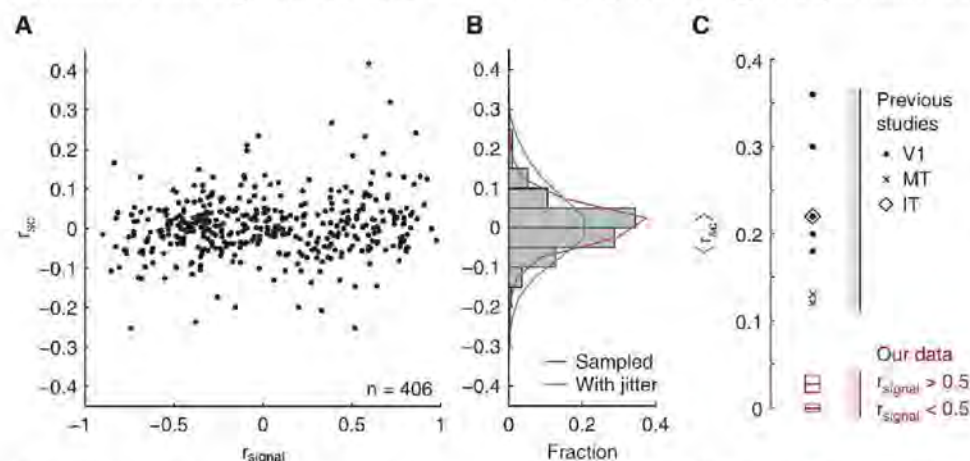
but not optimized for any specific cell. In total, we recorded 917 single units. After discarding cells that were not well isolated (>5% falsely assigned spikes), not visually responsive, or not tuned to orientation, we obtained 407 (D, 262; H, 145) single units. This corresponds to 1907 (D, 1335; H, 572) simultaneously recorded pairs, in 406 of which (D, 361; H, 45) both neurons were recorded by the same tetrode.

Neurons recorded from one tetrode are physically close to each other, have highly overlapping receptive fields, and are believed to receive strong common input. Nevertheless, spike count correlations in pairs of neurons recorded by the same tetrode were exceedingly low ( $r_{sc} = 0.005 \pm 0.004$ ; mean  $\pm$  SEM) (Fig. 2). Even cells with similar preferred orientations ( $r_{signal} > 0.5$ ) had very weak correlations ( $r_{sc} = 0.028 \pm 0.010$ ). This also held if pairs were strongly driven by gratings with orientations close to the cells' preferred orientations. Under such stimulation, spike count correlations were not larger than those under stim-

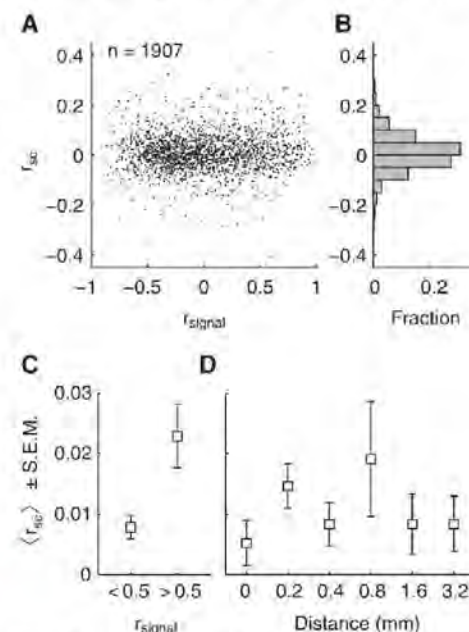
ulation with less optimal gratings ( $r_{sc} = 0.021 \pm 0.013$  versus  $0.016 \pm 0.011$ , two-sample  $t$  test:  $P = 0.80$ ,  $n = 361$ ). Only ~14% of all pairs with cells recorded from the same tetrode had correlations significantly different from zero ( $\alpha = 0.05$ ; for  $r_{signal} > 0.5$ : 13.2% positive, 2.4% negative; for  $r_{signal} < 0.5$ : 5.9% positive, 7.4% negative). Theoretical considerations and numerical studies indicate that much of the scatter in the distribution (Fig. 2B) may result from estimating correlation coefficients from finite data (figs. S2 and S3). Even though there were cases where similarly tuned neurons were correlated, these constituted only a small minority of pairs. Under our experimental conditions, spike count correlations for local ensembles were smaller than previously reported by more than an order of magnitude (Fig. 2C).

Previous studies report high correlations also for similarly tuned cells recorded on different electrodes separated by up to several millimeters (7–11). Therefore, we analyzed all simultaneously recorded pairs, including those pairs where the

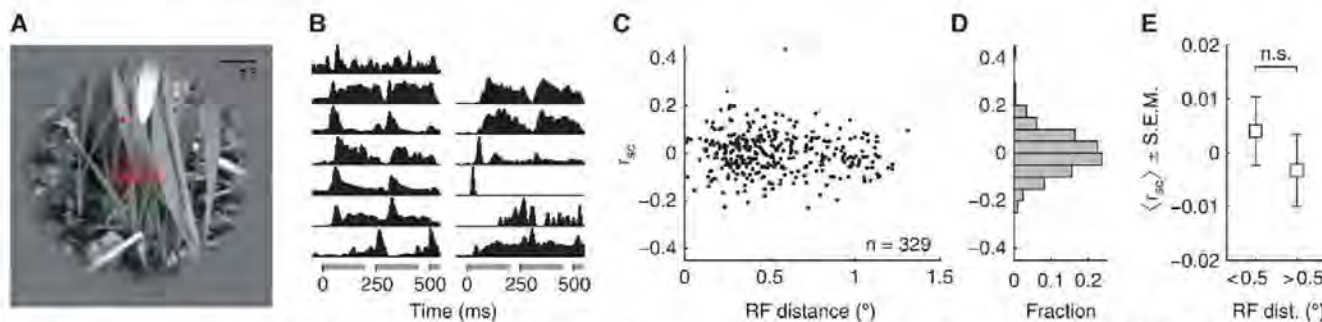
two neurons were recorded by different tetrodes. Average spike count correlations were low ( $r_{sc} = 0.010 \pm 0.002$ , mean  $\pm$  SEM) (Fig. 3, A and B). There was only a weak relation between tuning similarity and spike count correlation (two-sample  $t$  test,  $r_{signal} < 0.5$  versus  $r_{signal} > 0.5$ :  $P = 0.003$ ,  $n = 1907$ ) (Fig. 3C), and even similarly tuned cells had an average correlation close to zero ( $r_{signal} > 0.5$ :  $r_{sc} = 0.023 \pm 0.005$ , mean  $\pm$  SEM) (Fig. 3C). Correlations did not depend on the distance between the two neurons (linear regression slope:  $P = 0.99$ ; two-sample  $t$  test within versus across tetrodes:  $P = 0.16$ ) (Fig. 3D). Although there was a weak relation between two neurons' average firing rate and their spike count



**Fig. 2.** Spike count correlations of pairs of neurons recorded by the same tetrode. (A) Relation between  $r_{signal}$  and  $r_{sc}$  for all pairs of nearby neurons (both neurons recorded by the same tetrode). (B) Distribution of  $r_{sc}$  (mean  $\pm$  SEM =  $0.005 \pm 0.004$ ). Colored lines are distributions obtained by generating artificial data with the same number of trials as in the experiments (red, fixed  $r_{sc} = 0.01$ ; blue, average  $r_{sc} = 0.01$  and S.D. 0.1), which indicate that most of the scatter in the empirical distribution is due to estimating correlations from finite data [see supporting online material (SOM) text sections 1 and 2 for discussion]. (C) Average  $r_{sc}$  compared with previously reported values [black symbols, V1 (3, 4, 7–9); MT (5, 6); IT (2)]. Cells with similar tuning ( $r_{signal}$  of  $>0.5$ ) have slightly higher correlations ( $r_{sc} = 0.028 \pm 0.010$ ) than dissimilar cells ( $r_{sc} = -0.001 \pm 0.004$ , two-sample  $t$  test,  $P = 0.002$ ,  $n = 406$ ), but  $r_{sc}$  is an order of magnitude smaller than in previous reports.



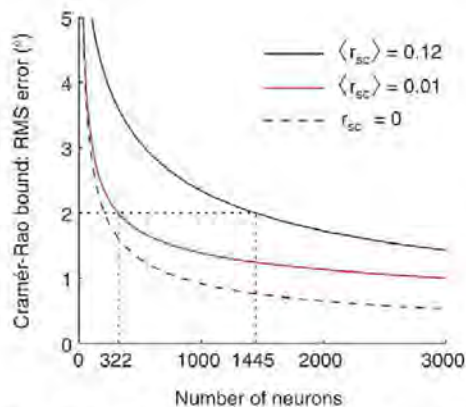
**Fig. 3.** (A) Relation between  $r_{signal}$  and  $r_{sc}$  for all pairs of simultaneously recorded neurons. (B) Distribution of  $r_{sc}$  (mean  $\pm$  SEM =  $0.010 \pm 0.002$ ). (C) Cells with similar tuning ( $r_{signal}$  of  $>0.5$ ) have slightly higher correlations ( $r_{sc} = 0.023 \pm 0.005$ ) than the remaining cells ( $r_{sc} = 0.008 \pm 0.002$ , two-sample  $t$  test,  $P = 0.025$ ,  $n = 1907$ ). (D)  $r_{sc}$  values do not depend on the distance between neurons (linear regression:  $P = 0.99$ ,  $n = 1907$ ). The bin at zero contains neurons recorded by the same tetrode (Fig. 2). Error bars show SEM.



**Fig. 4.** Spike count correlations for natural images. (A) Example of the stimulus with receptive field outlines of 13 simultaneously recorded neurons. Red dot, fixation spot. (B) Peristimulus time histograms of cells in (A) for this stimulus. In each trial, the stimulus was flashed four times for 200 ms, with 50-ms pauses. For consistency with the grating stimulus, spike counts were computed

from the first 500 ms (two stimulus flashes). (C) Relation between receptive field distance and  $r_{sc}$  is not significant (linear regression:  $P = 0.11$ ,  $n = 329$ ). (D) Distribution of  $r_{sc}$  (mean  $\pm$  SEM =  $0.001 \pm 0.005$ ). (E) Average  $r_{sc}$  for pairs with overlapping receptive fields versus pairs with nonoverlapping receptive fields. Two-sample  $t$  test:  $P = 0.89$ ,  $n = 329$ .





**Fig. 5.** Impact of correlation strength on encoding accuracy of neural populations. The Cramér-Rao bound (minimum achievable decoding error) is shown for different correlation structures: a previous report [black, refs. (5, 6)], our data (red), and an independent population of neurons (black dashed). Dotted lines highlight the number of neurons that would be necessary to achieve a decoding error of 2°. For details, see fig. S8 and SOM text, section 6.

correlations, this relation arose on time scales longer than one trial and therefore appears to be unrelated to shared presynaptic noise (fig. S4).

To investigate whether low correlations also occur under more naturalistic stimulus conditions, we conducted additional experiments in one of the monkeys (H). We first mapped the neurons' receptive fields before presenting natural images (Fig. 4, A and B) (25). The average  $r_{sc}$  was close to zero ( $r_{sc} = 0.001 \pm 0.005$ , mean  $\pm$  SEM, one-sample  $t$  test:  $P = 0.89$ ,  $n = 329$ ) (Fig. 4, C and D), with no relation between receptive field overlap and spike count correlations (linear regression slope:  $P = 0.12$ ,  $n = 329$ ) (Fig. 4C). Neurons with receptive fields within  $0.5^\circ$  of visual angle had spike count correlations similar to neurons with more distant receptive fields (two-sample  $t$  test:  $P = 0.43$ ,  $n = 329$ ) (Fig. 4E).

We recorded another 56 pairs of neurons while a third monkey (B) was presented with moving bars. As with the other stimuli, spike count correlations were close to zero under these conditions ( $0.014 \pm 0.011$ , mean  $\pm$  S.E.M.,  $P = 0.21$ ,  $n = 56$ ) (fig. S5).

Under a variety of stimulation conditions ranging from classic stimuli (such as bars and gratings) to natural images, spike count correlations in the primary visual cortex of awake monkeys were extremely low. These results stand in contrast to a number of previous studies, which report correlations of the order 0.1 to 0.3. Above we suggested four factors that could lead to spike count correlations unrelated to shared presynaptic noise (27). We now demonstrate how artificially introducing any one of these into our data produces correlations similar to previously published results. First, uncontrolled external or internal variables can be studied by assuming the neurons' firing rates are gain-modulated by a common underlying process. Shared modulations of only  $\sim 15\%$  can lead to correlations on the order of 0.2, as

shown in fig. S6. It is extremely hard, if not impossible, to control precisely the effects of attentional state, reward expectancy, task-solving strategy, or other cognitive factors (28). In contrast to extrastriate areas like V4 or MT, area V1 is much less affected by such modulations. Second, slow drifts over time or abrupt movements of the electrode tip can lead to changing waveforms and, thus, lost spikes or increased contamination by multiunit activity because of decreasing signal-to-noise ratio. Because movement is likely to affect all neurons recorded by one electrode, it can be modeled as a common gain modulation, and the above arguments apply. Our recordings were extraordinarily stable, as demonstrated by our ability to track neurons over several days (24). Third, contamination of waveform clusters identified as single units by spikes of other cells can create artificially high correlations and can even give rise to or amplify the limited-range correlation structure (fig. S7 shows that  $\sim 10\%$  false assignments during spike sorting can produce correlations of order 0.1). Fourth, during anesthesia, up and down states or even subtle variations in the level of anesthesia will inevitably cause changes in firing rates common to many cells [analogous to point 1 but on a relatively rapid time scale; see also (29)], potentially having a stronger impact on nearby cells. Thus, any meaningful characterization of the impact of noise correlations on population coding critically depends on the ability to obtain stable recordings from large populations of well-isolated adjacent neurons, ideally in an awake animal and in a cortical region like V1, which is not modulated strongly by variables that cannot be precisely controlled. Interpreting spike count correlations in terms of their effects on encoding capacities of cortical microcircuits or drawing conclusions about functional connectivity only makes sense if one can separate covariability because of uncontrolled variables from that reflecting intrinsic noise in the circuit.

Our findings have implications for models of cortical circuit architecture. The current view on the generation of correlations in cortical circuits rests on two major assumptions: (i) nearby cortical neurons receive a substantial amount of common input (6, 17, 30, 31); (ii) such common input leads to correlations (15–17, 32). In light of our data, at least one of these assumptions cannot be correct.

Based on measured spike count correlations, an influential modeling study inferred that, on average, nearby cells share up to 30% of their inputs (17). Under the same model, our data suggest that at most, 5% of the inputs are shared. Note that anatomical studies report  $\sim 10\%$  common inputs for excitatory neurons (30, 31). In addition, cortical excitatory connections may be very precisely structured (33) to form many independent subunits. In this case, most recorded pairs consist of neurons belonging to different subunits, and average correlations are very low.

Assumption (ii) has been challenged by recent network models in which a dynamic

balance of excitatory and inhibitory fluctuations counteracts correlations induced by common inputs (29, 34). This results in correlations that are positive on average but very low ( $\sim 0.01$ ), a prediction in good agreement with our data. To prevent small correlations from accumulating and dominating network activity, such a decorrelation mechanism might be a crucial prerequisite of hierarchical cortical processing.

Whatever the mechanism behind the decorrelated state of the neocortex, it offers substantial advantages for information processing: Consider a downstream neuron reading out the orientation of a grating from the activity of V1 neurons. If correlations were  $\sim 0.12$  on average (5, 6), the number of neurons necessary to achieve 2° precision (root mean square error) would be five times larger than those in the scenario in which the average correlations are  $\sim 0.01$  (Fig. 5 and fig. S8). Moreover, it is unclear whether neurons have access to the correlation structure of their synaptic inputs. If the network is in the decorrelated state, however, the effect of not taking any remaining correlations into account is small, and decoding is greatly simplified.

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small stimuli, barely covering the neurons' receptive fields, are used.

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### Supporting Online Material

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 Materials and Methods  
 SOM Text  
 Figs. S1 to S9  
 References

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# The Asynchronous State in Cortical Circuits

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Correlated spiking is often observed in cortical circuits, but its functional role is controversial. It is believed that correlations are a consequence of shared inputs between nearby neurons and could severely constrain information decoding. Here we show theoretically that recurrent neural networks can generate an asynchronous state characterized by arbitrarily low mean spiking correlations despite substantial amounts of shared input. In this state, spontaneous fluctuations in the activity of excitatory and inhibitory populations accurately track each other, generating negative correlations in synaptic currents which cancel the effect of shared input. Near-zero mean correlations were seen experimentally in recordings from rodent neocortex *in vivo*. Our results suggest a reexamination of the sources underlying observed correlations and their functional consequences for information processing.

The spiking activity of neurons is often correlated within local cortical populations (1–4). Although correlations could be a signature of active information processing (5, 6), they can also impair the estimation of information conveyed by the firing rates of neural populations (7, 2, 8) and might limit the efficiency of an organism for performing sensory discriminations (7, 2). Under special conditions, correlated spiking is an inevitable consequence of shared presynaptic input (9, 10). In general, however, the overall contribution of shared input to correlation magnitudes measured *in vivo* is unclear, as measured correlations could reflect mostly covariations in activity due to cognitive or external variables outside the control of the experimenter (11–13). To investigate the relation between correlations and shared input, we studied theoretically the correlation structures characteristic of densely connected recurrent networks.

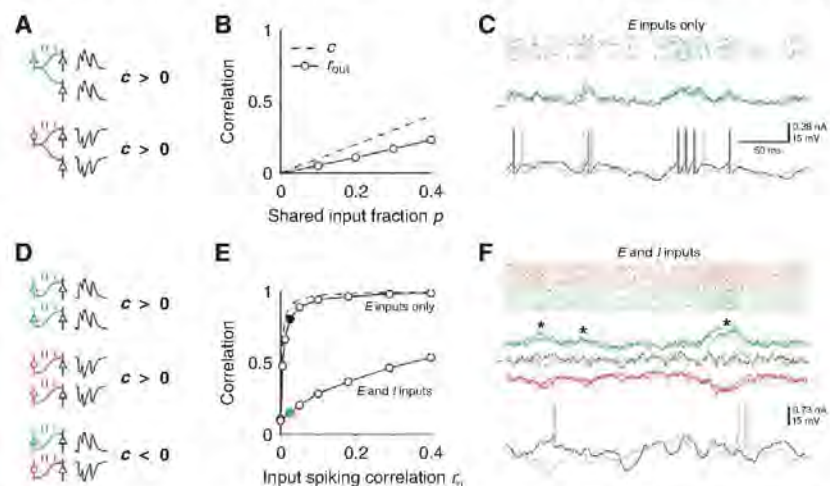
We start by considering how the correlation between a single neuronal pair depends on the

fraction  $p$  of shared inputs and the degree  $r_{in}$  to which the inputs are themselves correlated. The effect of shared input can be isolated by considering presynaptic neurons that fire independent-

ly ( $r_{in} = 0$ ). Both excitatory ( $E$ ) and inhibitory ( $I$ ) shared inputs cause positive correlations of a moderate magnitude in the synaptic input and spiking activity of the postsynaptic pair (Fig. 1, A and B) (9, 14). Spiking correlations  $r_{in}$  between inputs, however, have a major impact on the output correlation  $r_{out}$  of the postsynaptic pair. When all inputs are  $E$ , weak input correlations give rise to strongly correlated synaptic currents and output spikes (Fig. 1C). This occurs because, when  $p$  and  $r_{in}$  are small, the correlation  $c$  of the two input currents is approximately equal to

$$\bar{c} \approx p + Nr_{in} \quad (1)$$

(15), where  $N$  is the number of synaptic inputs, resulting in a large gain in the relation between  $r_{in}$  and  $r_{out}$  (Fig. 1E, upper solid curve). The situation changes when both neurons receive  $I$  as well as  $E$  inputs. Correlations between  $E$  or between  $I$  neurons lead to strongly cor-



**Fig. 1.** Effect of shared inputs and correlated inputs on output correlation. (A) Shared excitatory ( $E$ , green) or inhibitory ( $I$ , red) inputs induce positive correlations in the synaptic currents of two cells ( $c > 0$ ). (B) Correlation coefficient of synaptic currents  $c$  (dashed line) and output spikes  $r_{out}$  (circles, count window 50 ms) of a postsynaptic pair of integrate-and-fire neurons as a function of the shared input fraction  $p$  (21). Each postsynaptic cell received  $N_E = 250$  Poisson input spike trains. (C) Input spike raster (top), synaptic currents (middle), and membrane potentials (bottom) of a postsynaptic pair receiving weakly correlated  $E$  inputs [black circle in (E),  $r_{in} = 0.025$ ]. (D) Whereas correlations between  $E$  inputs or between  $I$  inputs contribute positively to  $c$ , correlations between  $E$  and  $I$  inputs have a decorrelating effect. (E) Correlations  $c$  (dashed line) and  $r_{out}$  (circles) as a function of the input spike correlation  $r_{in}$  at fixed  $p = 0.2$ .  $E$  inputs only: Each cell receives  $N_E = 250$  correlated Poisson spike trains (21);  $E$  and  $I$  inputs:  $N_I = 220$  inhibitory input trains were added with identical statistics and correlations. (F) Same as (C) but for the case with  $E$  and  $I$  inputs [blue circle in (E),  $r_{in} = 0.025$ ].  $E$  and  $I$  currents are shown separately from the total currents (black and gray). Asterisks indicate large fluctuations in the excitatory and inhibitory currents that occur simultaneously.

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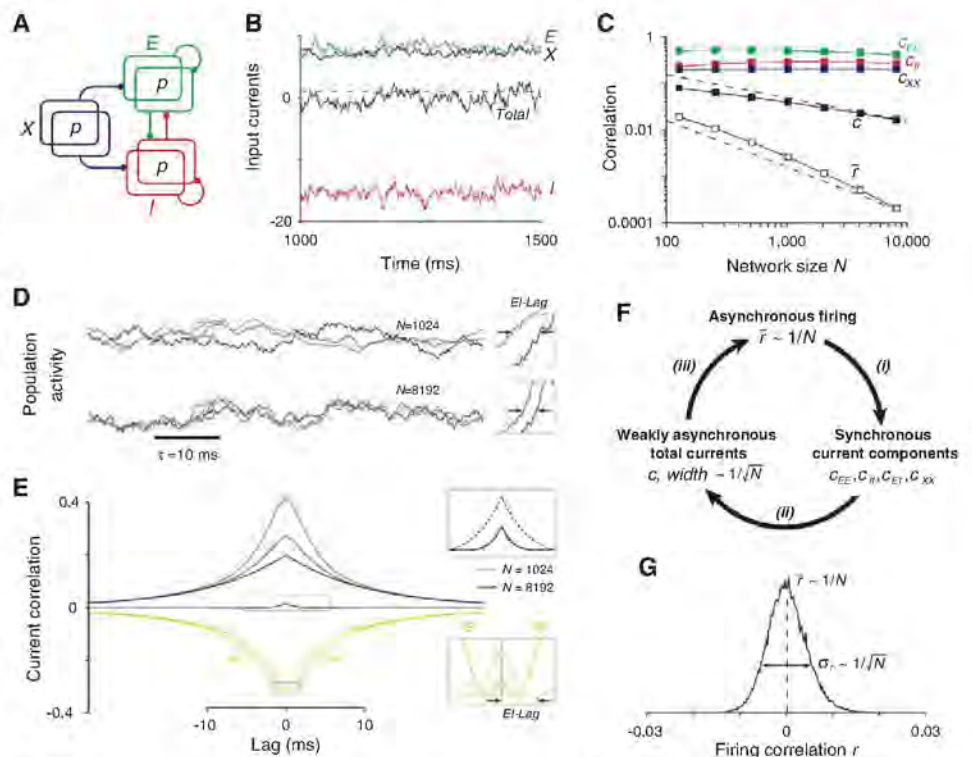
related excitatory and inhibitory synaptic currents (Fig. 1F, red and green traces). However, when  $E$  and  $I$  inputs are themselves correlated, large fluctuations in the excitatory and inhibitory currents occur simultaneously (Fig. 1F, asterisks) and cancel, which leads to a significant reduction in the correlation of the total synaptic currents  $c$  and output spikes (Fig. 1F, black and gray traces). Correlations between  $E$  and  $I$  inputs thus decorrelate the synaptic currents to postsynaptic neurons (16).

To investigate whether such decorrelation can arise spontaneously from the dynamics of a recurrent network, we characterized the behavior of correlations in a simple recurrent circuit of binary neurons (17–19). The network consists of two populations (of size  $N$ ) of  $E$  and  $I$  neurons connected randomly, both receiving excitatory projections from an external ( $X$ ) population of  $N$  cells (Fig. 2A). The network has two key properties: First, the connectivity is “dense” so that the connection probability  $p$  (and thus the mean fraction of shared input) is fixed independently of the network size [e.g.,  $p = 0.2$  (20) as in Fig. 2]. Second, the synaptic couplings are “strong,” such that only a small fraction of a cell’s excitatory inputs is enough to evoke firing (Fig. 2B); in the model, although the average number of inputs is proportional to  $N$ , the number of  $E$  inputs needed to induce firing is only proportional to  $\sqrt{N}$  (19). Our analysis showed that, even in the presence of shared input, the network settles into a stationary state in which the population-averaged firing correlation  $\bar{r}$  is weak, if inhibition is sufficiently strong and fast. In fact, in networks of different sizes,  $\bar{r}$  decreases in a way inversely proportional to  $N$  (Fig. 2C, open squares) [(17) section 2.3], a signature of asynchronous networks (18). In an asynchronous state, the variance of the population-averaged instantaneous activity scales in the same way as if the neurons were completely independent—as  $1/N$ . Thus, correlations in the asynchronous state do not qualitatively constrain averaging of activity across neural populations (fig. S2) (18).

Asynchronous activity persists in the presence of shared input because of a spontaneously generated tracking of fluctuations in the population-averaged instantaneous activities  $m_E(t)$  and  $m_I(t)$  of the  $E$  and  $I$  neurons (Fig. 2D) [(17) section 2.4]. Specifically,  $m_I(t)$  tracks  $m_E(t)$  with a small lag ( $EI$ -Lag), and they both closely follow the external instantaneous activity  $m_X(t)$ . In larger networks, tracking becomes more accurate and becomes perfect in the large  $N$  limit,

$$m_E(t) = A_E m_X(t); m_I(t) = A_I m_X(t) \quad (2)$$

where  $A_E$  and  $A_I$  are constants that depend on the network architecture. Tracking occurs because, when the connectivity is strong and dense, even small “random” fluctuations in instantaneous excitatory activity, of order  $1/\sqrt{N}$ , are large enough to recruit inhibitory feedback.



**Fig. 2.** Asynchronous activity in a binary recurrent network. (A) Schematic of the network architecture. The shared input fraction is  $p$ . (B) Strong coupling produces irregular spiking activity because of a dynamic balance between the large excitatory ( $E$  and  $X$ ) and inhibitory ( $I$ ) currents to each cell (19, 22). Dashed line represents threshold. (C) Population-averaged correlation coefficients of the firing activity ( $\bar{r}$ , open squares); total current ( $c$ , filled squares); and current components versus network size  $N$ . Dashed lines show  $1/\sqrt{N}$  and  $1/N$  scaling for comparison. (D) Instantaneous population-averaged activities (transformed to  $z$  scores) of the  $E$ ,  $I$ , and  $X$  neurons showing that tracking becomes more accurate with increasing  $N$ . (Insets) Instances of the lag between  $E$  and  $I$  activities ( $EI$ -Lag). Color code as in (B). (E) Population-averaged CCGs of the current components ( $N = 8192$ ). Color code as in (C). (Insets) Magnification of the peak of the  $IE$  and  $EI$  CCGs (bottom) shows that the  $EI$ -Lag decreases with  $N$ , which leads to the decrease in the magnitude and width of the total current CCG (top). (F) Description of the asynchronous self-consistent solution (see text). (G) The histogram of firing correlations in the network ( $EE$  pairs;  $N = 8192$ ) is wide:  $\sigma_r \gg \bar{r}$ .

Tracking of the instantaneous population activities is equivalent to a precise cancellation of the different components of the (zero-lag) population-averaged current correlation  $c$  (Fig. 2E) [(17) section 2.5]. Because the synaptic current to each cell consists of an excitatory and an inhibitory component, the average current correlation across cell pairs,  $c$ , can be decomposed into  $c_{EE}$ ,  $c_{II}$ , and  $c_{EI}$  (the term  $c_{IE} = c_{EI}$ ). Both  $c_{EE}$  and  $c_{II}$  are positive and large, i.e., independent of  $N$ , for large networks (Fig. 2C, colored squares) because of amplification of weak firing correlations and of shared  $E$  or  $I$  inputs (Fig. 2F,  $i$ ; and Fig. 1F, red and green traces). However,  $c_{EI}$  is large and negative because of correlations between  $E$  and  $I$  cells generated by tracking, which leads to the cancellation:

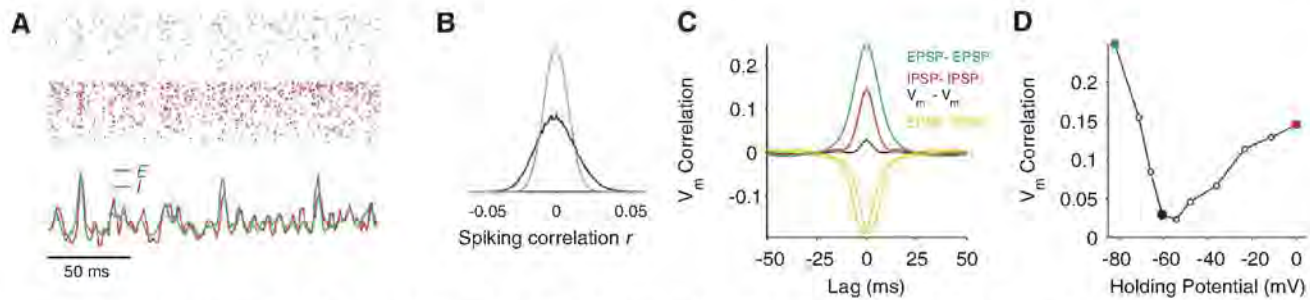
$$c = c_{EE} + c_{II} + 2c_{EI} \sim 1/\sqrt{N} \quad (3)$$

(Fig. 2C, filled squares; 2F,  $ii$ ; and fig. S3). Even after this cancellation, however, the instantaneous current correlation  $c$  is still larger than the correlation in firing  $\bar{r}$  (Fig. 2C, filled and open squares). This is possible because neurons in-

tegrate their inputs over time, so that the instantaneous correlation  $\bar{r}$  is related to the area under the current cross-correlogram (CCG). Because tracking becomes faster for larger networks, both the width of the current CCG (effectively set by the  $EI$ -Lag) and its magnitude decrease as  $1/\sqrt{N}$  (Fig. 2E, insets). Its area is thus  $1/N$ , as required for asynchronous firing (Fig. 2F,  $iii$ ). Because the asynchronous state just described is a dynamical phenomenon, it does not require fine-tuning of network parameters (fig. S4). Parameter changes lead to adjustments in rates and correlations such that the cancellation in Eq. 3 still holds.

Although the theory predicts that the population-averaged correlation  $\bar{r}$  should be close to zero, it does not predict that every pair of cells should be as weakly correlated. Rather, the distribution of  $r$  across pairs is “wide,” with a standard deviation  $\sigma_r$  much larger than its mean  $\bar{r}$  for large networks ( $\sigma_r$  decays only as  $1/\sqrt{N}$ ), which results in similar numbers of positively and negatively correlated pairs (Fig. 2G). This is because the hard-wired sources of correlation have a strong impact on individual  $r$  values (of order  $1/\sqrt{N}$ ) and

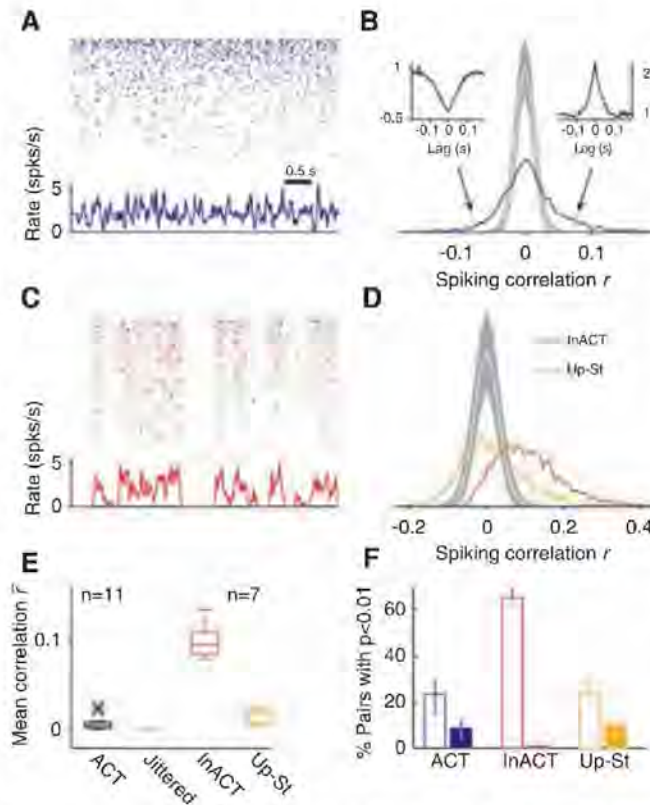




**Fig. 3.** Cancellation of correlations in a recurrent network of spiking neurons. **(A)** Raster (top) of 500 *E* (green) and *I* (red) neurons sorted by rate in a conductance-based integrate-and-fire network receiving shared independent Poisson inputs ( $p = 0.2$ ). Bottom curves show tracking of instantaneous population-averaged activities (transformed to *z* scores, bin size 3 ms). Average firing rates of *E* and *I* cells were 1 and 3.6 spike per s, respectively. **(B)** Histogram of spike count correlations (black; count window 50 ms) and

of jittered spike trains [gray, jitter  $\pm 500$  ms (21)]. **(C)** Population-averaged CCGs of the membrane potential containing mostly EPSPs (green) or IPSPs (red) in both cells, or EPSPs for one cell and IPSPs for the other (gold). The black curve is from pairs at resting potential. **(D)** Peak height of the membrane potential CCG as a function of the mean holding potential of both neurons in the pair. Green and red circles correspond to the reversal of inhibition and excitation, and the black circle corresponds to rest.

**Fig. 4.** Distribution of correlations in the rat neocortex in vivo. **(A)** Raster (top) and instantaneous population activity (bottom) for a population of 100 simultaneously recorded neurons (sorted by rate) during a period of cortical activation (ACT). **(B)** Histogram of spike count correlations of the population in (A) is wide ( $\sigma_r \gg \bar{r}$ ). The white curve is the mean histogram of the jittered spike trains [jitter  $\pm 200$  ms, gray shade 95% confidence interval; count window 50 ms (21)]. Insets show average raw cross-correlograms of all negatively (left) and positively (right) significantly correlated pairs ( $P < 0.01$ ). **(C)** Same as (A) and (B) for the same population of cells during a period of cortical inactivation (InACT). Histogram of correlations during InACT is biased toward positive values (red). Restricting the analysis to up-state activity by removing down-state periods [black brackets in (C), (21)] largely eliminates the positive bias (Up-St, orange). **(E)** Box-whisker plots showing the distribution of mean correlations across experiments for different conditions. Crosses represent outliers. **(F)** Median fraction of significantly correlated pairs ( $P < 0.01$ , white bars) and of significantly and negatively correlated pairs (filled bars) across experiments. Error bars represent interquartile range.



therefore generate large heterogeneity across pairs (fig. S3). Asynchronous activity is also possible under nonstationary conditions: Numerical simulations with time-varying inputs display a similar correlation structure if  $r$  is computed with respect to the time-varying instantaneous average activity of each cell (fig. S5).

Active decorrelation of synaptic currents also occurs in a more biologically plausible network of spiking neurons. We simulated large networks of randomly connected conductance-based integrate-and-fire neurons (21), with parameters chosen to produce a balanced state (19, 22)

where neurons fired irregularly (Fig. 3A). As predicted, the distribution of spike count correlation coefficients  $r$  is wide (Fig. 3B), with an extremely low average (in *EE* pairs  $\bar{r} < 0.001$  for all count window sizes, fig. S6C). This happened for a large range of average firing rates and connection probabilities (fig. S7, A to F), with synchrony developing only when inhibition was substantially slower than excitation (fig. S7, G to I). The distribution of  $r$  conditioned on the response to time-varying external inputs was also wide for a large range of modulation frequencies (fig. S8). To determine

whether a cancellation between the components of the current correlation (Eq. 3) underlies the small value of  $\bar{r}$  observed, we injected different levels of constant current into cell pairs in which we had disabled the spiking mechanism. The range of current levels was adjusted to isolate the excitatory postsynaptic potential (EPSP) and the inhibitory postsynaptic potential (IPSP) components near their respective reversal potentials (23), or combinations of EPSPs and IPSPs at intermediate potentials. The correlation between isolated EPSPs (Fig. 3C, green) and between isolated IPSPs (red) was much larger than the correlation measured with no injected current (black), because of a cancellation with the large negative correlation between EPSPs and IPSPs (gold). The V-shaped relation between membrane potential correlation and holding potential (Fig. 3D) is an experimentally testable prediction of our theory.

The existence of a wide distribution of spike count correlations was confirmed in neuronal population recordings collected with silicon microelectrodes in somatosensory and auditory cortices of urethane-anesthetized rats (21). Under urethane anesthesia, cortical activity displays spontaneous changes in state (24) homologous to those seen during sleep (25). Network activity alternates between an “activated” (ACT) state of tonic firing, resembling that seen in rapid eye movement (REM) sleep (Fig. 4, A and B, blue), and an “inactivated” state (InACT), characterized by global fluctuations in population activity (up-down transitions) resembling slow-wave sleep (Fig. 4, C and D, red). During ACT periods, correlations were, on average, remarkably small, and the correlation histogram was wide. (Fig. 4B shows one experiment;  $\bar{r} = 0.0075$ ; 47% of pairs negatively correlated.) These values were typical of ACT state correlations across different animals [(Fig. 4E)  $n = 11$  recording sessions in nine rats,  $\bar{r}$  median was 0.0053 (0.0024 to 0.0094 interquartile range); across all 30,772 pairs,  $\bar{r} = 0.0052$ , and 47% had  $r < 0$ ]. This behavior did not depend strongly on the time scale at which correlations were measured (fig. S9). Although  $\bar{r}$  in the ACT state was systematically low, it was positive and significantly different from zero



in all experiments (Fig. 4E;  $P < 0.005$ ). A minority of both positive and negative correlations were statistically significant (Fig. 4, B and F, blue versus gray). Pairs with significant negative (positive) correlations showed clear troughs (peaks) in their cross-correlograms on average (Fig. 4B, insets, and fig. S10). Finally, the correlation histogram during the ACT state was still wide, even if only neurons recorded in the same shank were considered (fig. S11). During InACT periods, as expected from comodulation by the slow oscillation, the distribution of  $r$  was consistently biased toward positive values [(Fig. 4, C to F, red) for seven sessions in five rats,  $\bar{r}$  median was 0.0953 (0.088 to 0.109 interquartile range); across all 18,916 pairs,  $\bar{r} = 0.096$ , and 9% had  $r < 0$ ]. Within up-state periods, however, average correlations were again weak (26); removal of down-states from the recorded spike trains (21) resulted in correlation histograms similar to those during the ACT state [(Fig. 4, D and E, orange)  $\bar{r}$  median was 0.0163 (0.0066 to 0.023 interquartile range); across all 18,916 pairs,  $\bar{r} = 0.0136$ , and 45% had  $r < 0$ ]. Thus, correcting for common modulations in activity revealed a wide distribution of correlations even under nonstationary conditions in vivo.

In summary, we demonstrate theoretically that recurrent network dynamics can lead to an active decorrelation of synaptic currents, resulting in a state of arbitrarily low mean correlation. We therefore conclude that shared input does not inevitably cause correlated activity. By preventing uncontrolled network-wide synchrony, this mechanism generates a background of weakly correlated spiking, as required for efficient information processing based on either firing rates or coordinated spike timing patterns (27, 28). Both simulations and in vivo recordings showed a wide distribution of correlations under stationary conditions. In nonstationary conditions,

global activity modulations can result in positively biased correlations, but correlations around the mean activity imposed by these modulations can still be extremely small (Fig. 4, D and E; and figs. S5 and S8). Similarly weak correlations have been reported in visually driven neural populations in area V1 of awake behaving monkeys (29). However, as the constellation of inputs driving a cortical circuit is, in general, unknown to the experimenter, positive correlations may persist even after all experimentally controlled variables are accounted for (11, 12). Whether “residual” correlations of this nature will have a strong impact on coding will depend on the extent to which downstream networks are able to disambiguate modulations in activity due to different sources. In either case, we suggest that cortical circuitry does not itself constitute an irreducible source of “noise.”

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amount  $r_{in}$ , out of which  $Np$  are common, is equal to  $[p + r_{in}(N-p)]/[1 + r_{in}(N-1)]$ , which is approximately equal to  $p + Nr_{in}$  when  $p \sim r_{in}N \ll 1$ .

16. When the firing statistics of the  $E$  and  $I$  populations are identical, the leading order effect of positive firing correlations on  $c$  can only be  $\geq 0$ . If excitation and inhibition are precisely balanced, the equality is realized and the fraction of shared input sets the population-averaged firing correlation (9).
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### Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S11

References

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## Direct Restart of a Replication Fork Stalled by a Head-On RNA Polymerase

Richard T. Pomerantz and Mike O'Donnell\*

In vivo studies suggest that replication forks are arrested by encounters with head-on transcription complexes. Yet, the fate of the replisome and RNA polymerase (RNAP) after a head-on collision is unknown. We found that the *Escherichia coli* replisome stalls upon collision with a head-on transcription complex, but instead of collapsing, the replication fork remains highly stable and eventually resumes elongation after displacing the RNAP from DNA. We also found that the transcription-repair coupling factor Mfd promotes direct restart of the fork after the collision by facilitating displacement of the RNAP. These findings demonstrate the intrinsic stability of the replication apparatus and a previously unknown role for the transcription-coupled repair pathway in promoting replication past a RNAP block.

In vivo studies suggest that replication forks are arrested by head-on transcription complexes, but are unaffected by codirectional transcription complexes (1) [supporting online material (SOM) Text S1]. Mechanisms that

resolve head-on collisions in favor of the replisome are therefore necessary for chromosome duplication and may preserve genomic integrity by preventing fork collapse. In vivo data indicate that head-on replisome–RNA polymerase

(RNAP) collisions cause chromosomal deletions, which suggests dissociation of the replisome (2). Genetic studies implicate recombinational repair in resolving conflicts between replication and transcription, which also suggests the possibility of fork collapse (3, 4). Similarly, in vitro data imply that the replisome dissociates after encountering a *lac* repressor, which arrests the fork (5). In contrast, several in vivo studies indicate that although replication forks stall at protein barriers, the replisome remains stable and resumes elongation after removal of the block (6). Thus, replisome stalling may not necessitate fork collapse (7). We investigated the stability of the *Escherichia coli* replisome after it encounters a head-on RNAP in vitro.

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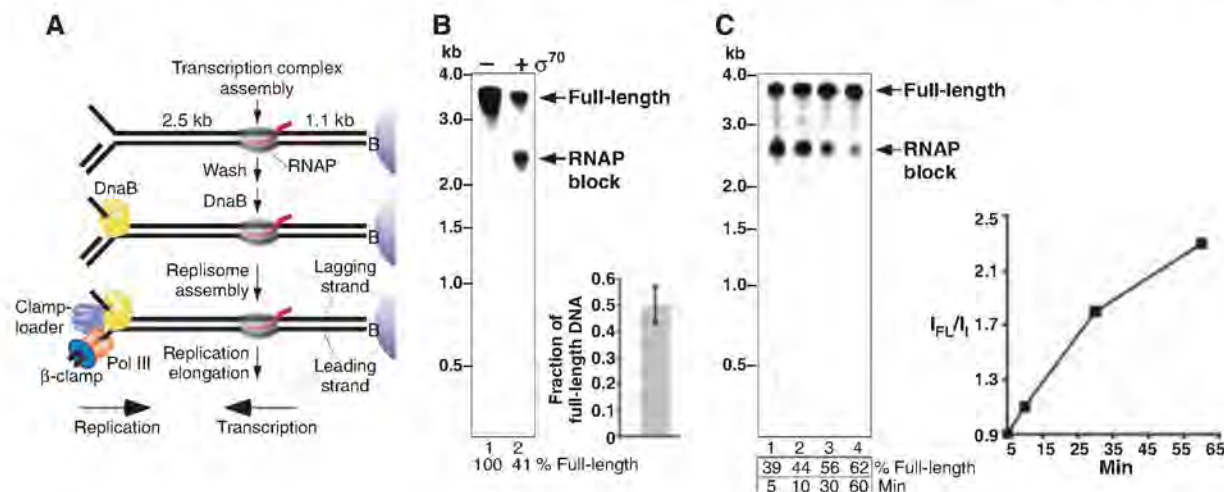
The *E. coli* replisome is a multiprotein complex that copies DNA with high speed [ $\sim 630$  nucleotides (nt)  $s^{-1}$ ] and processivity ( $\sim 50$  kb) (8). A solid-phase assay was used to study a replisome-RNAP head-on collision (Fig. 1A). An RNAP-halted elongation complex was assembled on linear DNA, immobilized to streptavidin beads, then washed with a high concentration of salt to remove unstable RNAP-DNA complexes (fig. S1) (9). Next, the replisome was assembled in two steps: First, the replicative DnaB helicase that encircles the lagging strand was added; second, DNA polymerase III (Pol III), the clamp loader, and the  $\beta$  clamp were added along with ATP (adenosine 5'-triphosphate), [ $^{32}P$ ]- $\alpha$ -dCTP (2'-deoxycytidine 5'-triphosphate), and [ $^{32}P$ ]- $\alpha$ -dGTP (2'-deoxyguanosine 5'-triphosphate). Fork movement was initiated by adding [ $^{32}P$ ]- $\alpha$ -dATP and [ $^{32}P$ ]- $\alpha$ -dTTP with single-strand DNA binding protein (SSB). We observed a 2.5-kb product equal to the distance from the fork to the promoter, indicating that the

replisome was impeded by the RNAP (Fig. 1B, lane 2). Full-length DNA (3.6 kb) was also produced, suggesting incomplete promoter occupancy by RNAP or replisome read-through of the transcription complex (Fig. 1B, lane 2). Omitting the promoter specificity factor,  $\sigma^{70}$ , resulted in only full-length DNA (Fig. 1B, lane 1). An average of 50% ( $n = 8$ ) of the replisomes produced full-length DNA in the presence of a head-on RNAP (Fig. 1B, right), which exceeded the number of templates that lacked RNAP (24%; fig. S2). This suggested that  $\sim 26\%$  of the replisomes passed a head-on RNAP during the 10-min time course. We determined whether the replisome reads-through RNAP directly by performing a pulse-chase experiment in which cold dNTPs (deoxynucleoside triphosphates) were added after 5 min, and extension of the 2.5-kb product was monitored (Fig. 1C). A steady increase in the ratio of full-length to intermediate-length product was observed, indicating that the replisome passes the RNAP, albeit after pausing

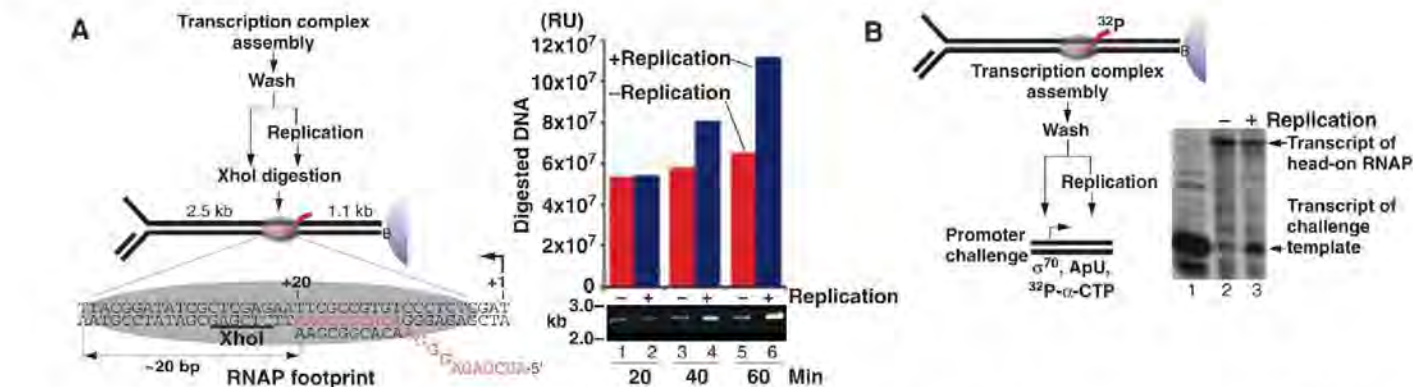
for a considerable duration (Fig. 1C). The relatively large amount of full-length product (39%) observed after 5 min suggests that some replisomes might have passed the RNAP with high efficiency. Replisome read-through of RNAP on a different template ruled out any sequence-specific effects (fig. S3). The stalled replisome remained active for 60 min after the collision without the need for primosomal proteins, such as PriA/C, that are necessary to reload DnaB onto SSB-coated single-strand DNA (Fig. 1C) (10, 11). DnaB therefore stayed bound to the stalled fork. Consistent with previous studies, we demonstrated that DnaB was required for replication and that SSB prevented DnaB loading in the absence of primosomal proteins (fig. S4). Thus, although the replication fork stalls upon encountering a head-on RNAP, the replisome remains intact and resumes elongation, presumably after displacing the transcription complex.

We determined whether the replisome displaces the RNAP from DNA by Xho I digestion

**Fig. 1.** The replisome slowly passes a head-on RNAP. (A) A head-on RNAP was assembled on immobilized DNA. DnaB was added, then the replisome was assembled and replication initiated. Replisome component functions ( $\beta$ ): Pol III core (orange), synthesizes DNA;  $\beta$  clamp (dark blue), confers processivity to Pol III; clamp-loader (light blue), assembles  $\beta$  clamps onto primed sites; DnaB (yellow), unwinds DNA. (B) Leading-strand synthesis was performed in the presence (lane 2) or absence (lane 1) of a head-on RNAP. (Right) The mean fraction ( $\pm$  SE) of full-length DNA (0.5,  $n = 8$ ) produced in the presence of a head-on RNAP is plotted. (C) Time course of leading-strand



synthesis on DNA containing a head-on RNAP. The ratio of full-length to intermediate-length DNA ( $I_{FL}/I_I$ ) is shown in the right plot ( $I_{FL}$ , intensity of full-length DNA;  $I_I$ , intensity of intermediate-length DNA).

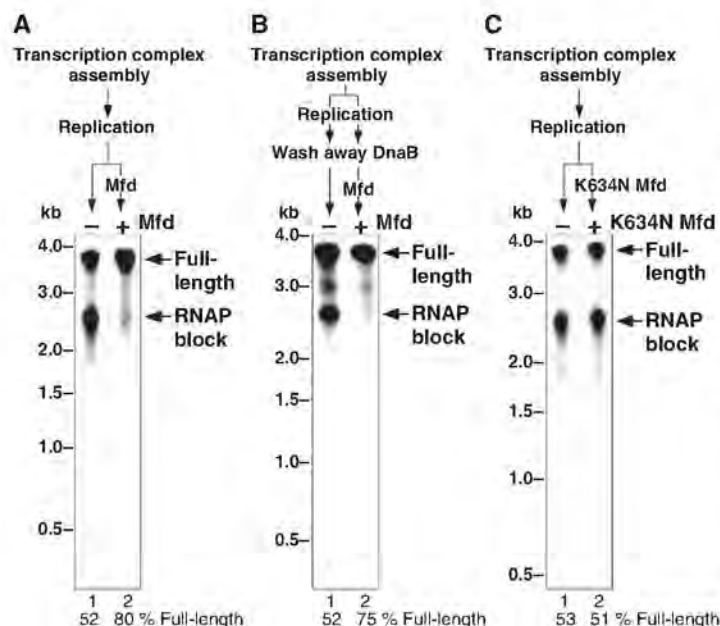


**Fig. 2.** The replisome displaces a head-on RNAP from DNA. (A) Replisome displacement of RNAP was probed by Xho I digestion of the promoter-proximal sequence in the presence or absence of replication. A halted RNAP was assembled on DNA, and the reaction was divided. Replication was (lanes 2, 4, and 6; blue bars) or was not (lanes 1, 3, and

5; red bars) initiated, and samples were treated with Xho I at the indicated times. RU, relative units. (B) RNAP displacement in the presence (lane 3) or absence (lane 2) of replication was determined by monitoring transcription of a challenge template. Transcription of challenge template control (lane 1).



**Fig. 3.** Mfd promotes fork progression following a head-on collision. Leading-strand synthesis was performed on DNA containing a head-on RNAP. Reactions were divided before (B) or after (A and C) replication was performed for 5 min. (B) DnaB was removed from solution by washing after the collision was formed. Buffer (lanes 1) or either wild-type (A and (B), lane 2) or K634N Mfd (C, lane 2) was then added for a further 5 min.



of the promoter-proximal sequence, which is protected by RNAP (Fig. 2A). RNAP occupancy of the promoter blocks digestion, whereas RNAP displacement allows digestion. Removing ribonucleotides (NTPs) by washing prevents reassembly of the halted RNAP. A significant increase in digestion was observed only when replication was performed (Fig. 2A, right; compare blue and red bars). Little RNAP displacement was detected at 20 min, which is likely due to replication of only 8.5% of the DNA, probably as a result of RNAP binding to the primer-template (12) (fig. S5). Nevertheless, the large increase in digestion due to replication indicates that the replisome displaces the RNAP. Similar results were observed in the presence of GreB and limiting NTPs, which inhibit RNAP backtracking (fig. S6). We further investigated RNAP displacement by monitoring transcription of a challenge template in the presence of  $\sigma^{70}$  and limiting NTPs. Transcription of the challenge template was only observed after replication, which indicates that the replisome displaced the RNAP (Fig. 2B, compare lanes 2 and 3). These results are consistent with the ability of DnaB to displace a protein block during DNA unwinding (13).

Genetic data implicate RNAP modulators such as Mfd in resolving conflicts between replication and transcription (3). Mfd displaces a halted RNAP from DNA and recruits the nucleotide excision repair machinery to the site, which results in preferential repair of the transcribed strand when RNAP stalls at lesion—referred to as transcription-coupled repair (TCR) (14–18). TCR has been postulated to promote fork progression by displacing RNAP blocks from DNA (16). We examined whether Mfd facilitates restart of the fork after a head-on collision. After providing 5 min to allow the replisome to collide with RNAP, we divided the reaction and treated it with either Mfd or buffer for a further 5 min. Nearly full extension of the

2.5-kb product was observed upon addition of Mfd (Fig. 3A, compare lanes 1 and 2), which removed the RNAP block (fig. S7). We determined whether Mfd-mediated replication restart requires a supply of DnaB in solution (Fig. 3B). For this experiment, the beads were washed after the collision then resuspended in buffer containing dNTPs and all the replication proteins except DnaB either in the presence or absence of Mfd. The addition of Mfd again resulted in extension of the 2.5-kb product, providing further evidence that DnaB stays bound to the stalled fork. Next, the experiment of Fig. 3A was repeated with Mfd K634N, which is defective in ATP hydrolysis and can no longer dislodge RNAP (17). Mfd K634N failed to promote extension of the 2.5-kb product (Fig. 3C, compare lanes 1 and 2). These results indicate that Mfd promotes fork progression after the collision by using the energy of ATP, and hence translocase activity, to dissociate the transcription complex ahead of the stalled fork. Finally, we demonstrated that the ability of Mfd to promote replication past a head-on RNAP was unaffected by the addition of all four NTPs and GreB, which promote transcription elongation (fig. S8).

In conclusion, we find that although the replication fork stalls upon collision with a head-on transcription complex, the replisome remains stable and resumes elongation after displacing the RNAP (fig. S9, left). It is conceivable that the collision may induce RNAP backtracking. However, the lack of stimulation of RNAP endonuclease activity after the collision suggests that this is not the case (fig. S10). Moreover, the addition of GreB and NTPs, which inhibit backtracking, have no effect on our assays (figs. S6, S8, and S11). A previous study of the T4 replisome reported that a head-on RNAP remains bound to the DNA, but the RNAP and transcript switch strands as the replication fork passes (19). This result is difficult to reconcile with the

current view of transcription. We find that Mfd promotes direct restart of the fork following the collision by facilitating displacement of the RNAP (fig. S9, right). Codirectional collisions are resolved without auxiliary factors; the replisome uses mRNA as a primer to reinitiate leading-strand synthesis after displacing a codirectional RNAP from DNA (9). Pol III extension of the RNA was not observed in our study, probably due to displacement of the transcript. Genetic data suggest that recombinational repair and other RNAP modulators help resolve replisome-RNAP conflicts, thus explaining the normal growth rate of cells lacking Mfd (3). *mfd* cells, however, demonstrate a greater lapse in replication and cell growth following ultraviolet irradiation, which supports a role for Mfd in facilitating replication through transcription complexes arrested by lesions in vivo (20). Our data demonstrate a new role for TCR in promoting replication past an RNAP block and may have implications for human disorders that result from deficient TCR, such as Cockayne syndrome (15, 16).

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## Supporting Online Material

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Figs. S1 to S11

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# p53 Controls Radiation-Induced Gastrointestinal Syndrome in Mice Independent of Apoptosis

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Acute exposure to ionizing radiation can cause lethal damage to the gastrointestinal (GI) tract, a condition called the GI syndrome. Whether the target cells affected by radiation to cause the GI syndrome are derived from the epithelium or endothelium and whether the target cells die by apoptosis or other mechanisms are controversial issues. Studying mouse models, we found that selective deletion of the proapoptotic genes *Bak1* and *Bax* from the GI epithelium or from endothelial cells did not protect mice from developing the GI syndrome after sub-total-body gamma irradiation. In contrast, selective deletion of p53 from the GI epithelium, but not from endothelial cells, sensitized irradiated mice to the GI syndrome. Transgenic mice overexpressing p53 in all tissues were protected from the GI syndrome after irradiation. These results suggest that the GI syndrome is caused by the death of GI epithelial cells and that these epithelial cells die by a mechanism that is regulated by p53 but independent of apoptosis.

Concern over the potential exposure of civilians to radiation has increased interest in understanding the mechanisms underlying radiation injury. Acute radiation syndrome (also called radiation sickness) occurs when the body is exposed to a high dose of penetrating radiation in a short period of time. The constellation of symptoms that define acute radiation syndrome include effects related to bone marrow toxicity (hematopoietic syndrome) and gastrointestinal (GI) toxicity (the GI syndrome). The hematopoietic syndrome can occur after whole-body irradiation (WBI) at a dose of 6 Gy. The resultant reduction in leukocytes and platelets leads to infection or hemorrhage, respectively, and death can occur within 30 days of exposure (1). In certain situations, death from the hematopoietic syndrome can be prevented (for example, by shielding

part of the bone marrow or by a subsequent bone marrow transplant) (2–4). At higher doses of radiation, death occurs within 10 days from damage to the GI tract (3, 4). In contrast to the hematopoietic syndrome, no medical countermeasures are approved to prevent or treat the GI syndrome.

Despite decades of study, the critical cellular targets of radiation-induced toxicity in the GI tract and the molecular mechanism of cell death underlying the GI syndrome remain controversial (5). For example, the role of p53 in the GI syndrome is unclear. Radiation activates p53 in the GI epithelium (6), which induces PUMA to kill cells via the intrinsic pathway of apoptosis in 4 hours (7–10). Deletion of either p53 or PUMA blocks radiation-induced apoptosis of most intestinal epithelial cells. Because death after WBI is delayed in mice lacking PUMA, p53-mediated apoptosis has been implicated in regulating the GI syndrome (10). Other research groups have reported that the absence of p53 does not alter the GI syndrome and have proposed that it is instead regulated by radiation-induced apoptosis of endothelial cells (11). Although radiation-induced endothelial cell apoptosis can proceed in the absence of p53 or PUMA (10, 12), the intrinsic pathway is still believed to be involved because *Bax*<sup>−/−</sup> and *Bak1*<sup>−/−</sup> mice show reduced endothelial cell apoptosis after irradiation (13, 14).

Others have implicated p53 in protecting mice from the GI syndrome (15). Although radiation-induced apoptosis is suppressed in p53-null mice (6), other modes of cell death, such as mitotic catastrophe, can still occur (16). For example, 24 hours after radiation at doses of 8 Gy or more, reproductive or clonogenic cell death (hereafter referred to as mitotic death) occurs in the small intestine (17). The molecular

mechanism of this form of death remains unclear, but at this dose of radiation, a cell cycle arrest is followed by proliferation, in which some cells may undergo an aberrant mitosis and die by mitotic catastrophe (16, 18, 19).

To investigate the cellular target and the molecular mechanism of cell death in the acute radiation syndrome, we have used the Cre-loxP system (20). Mice in which Cre is expressed under the control of a tissue-specific promoter can selectively delete DNA flanked by loxP sites, which is termed a floxed (FL) allele, in a cell type-specific manner. Therefore, we used mice (*VillinCre*) that express Cre specifically throughout the GI epithelium under the control of the *Villin* promoter (21) and mice (*Tie2Cre*) in which Cre is expressed specifically in endothelial cells and the hematopoietic system under the control of the *Tie2* promoter (22). To investigate the role of the intrinsic pathway of apoptosis in the acute radiation syndrome, *VillinCre* or *Tie2Cre* mice were crossed with mice carrying floxed alleles for *Bax* (23). In some settings, BAK can mediate BAX-independent apoptosis via the intrinsic pathway (24). Therefore, experiments were carried out on a *Bak1*-null genetic background (25).

To determine whether *Bax* was deleted in the hematopoietic system in *Tie2Cre; Bak1*<sup>−/−</sup>; *Bax*<sup>FL/−</sup> mice, we subjected cell lysates from the spleen, thymus, and lymph nodes to Western blot analysis. In these tissues, the levels of BAX were significantly reduced when compared to *Tie2Cre; Bak1*<sup>−/−</sup>; *Bax*<sup>FL/+</sup> littermate controls (Fig. 1A). Deleting the intrinsic pathway of apoptosis from the hematopoietic system in *Tie2Cre; Bak1*<sup>−/−</sup>; *Bax*<sup>FL/−</sup> mice protected the bone marrow (fig. S1) and improved the survival of the mice after exposure to 12.5 Gy of WBI (Fig. 1B). Therefore, the intrinsic pathway of apoptosis is required for death from the hematopoietic syndrome. Because Cre is also expressed in endothelial cells in *Tie2Cre* mice, it is possible that protection of the vascular niche (26) within the bone marrow contributed to increased survival from WBI.

We next examined the role of the intrinsic pathway of apoptosis in the GI syndrome. For these experiments, the bone marrow of the front legs was shielded for sub-total-body irradiation (SBI) to avoid the hematopoietic syndrome (fig. S2). Initial experiments were performed with mice in which either *Bak1* or *Bax* was deleted. No difference in survival from the GI syndrome was observed for *Bak1*<sup>−/−</sup> mice versus *Bak1*<sup>+/+</sup> littermate controls or between *Bax*<sup>−/−</sup> mice and *Bax*<sup>+/+</sup> littermate controls after SBI (fig. S3). Because *Bak1*<sup>−/−</sup>; *Bax*<sup>−/−</sup> mice are generally not viable (23), we studied mice in which *Bak1* and *Bax* were deleted from either endothelial cells or GI epithelial cells.

To determine whether *Bax* was deleted from endothelial cells in *Tie2Cre; Bak1*<sup>−/−</sup>; *Bax*<sup>FL/−</sup> mice, endothelial cells were isolated from lungs by fluorescence-activated cell sorting. PECAM<sup>+</sup>, SCA-1<sup>+</sup> cells were collected (25) and analyzed

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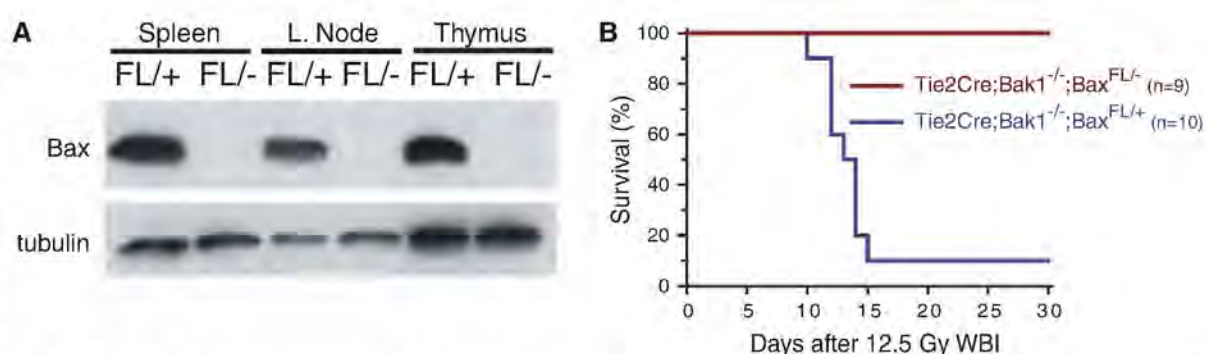
by Western blot for BAX (Fig. 2A). In these cells from *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice, BAX was not detected. Within the villi, apoptosis of mesenchymal cells, which includes endothelial cells and lymphocytes, occurs 4 hours after doses of radiation that cause the GI syndrome (11, 27). Therefore, we harvested the small intestine of mice 4 hours after 16.95 Gy of SBI and assessed mesenchymal cell apoptosis within the villi by TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling) staining. Deleting the intrinsic pathway of apoptosis in *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice significantly reduced mesenchymal cell apoptosis as compared to *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice that retained a wild-type *Bax* allele (Fig. 2, B and C) but did not improve survival from the GI syndrome after SBI (Fig. 2D and fig. S4). These results suggest that mesenchymal cell apoptosis within the intervillus region of the intestine does not initiate the GI syndrome.

We also evaluated the role of the intrinsic pathway of apoptosis in mediating the GI syndrome in GI epithelial cells. To determine whether BAX was deleted in the GI epithelium of *VillinCre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice, we isolated epithelial cells from the small intestine and analyzed them for BAX expression by Western blot (25). In *VillinCre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice, BAX was deleted from the GI epithelium (Fig. 3A). Although deleting the intrinsic pathway of apoptosis in the GI epithelium reduced apoptosis in the crypt epithelium fivefold after irradiation (Fig. 3, B and C), this did not improve survival after SBI (Fig. 3D and fig. S5). Taken together, these results suggest that survival from the GI syndrome is not determined by apoptosis of either endothelial cells or GI epithelial cells.

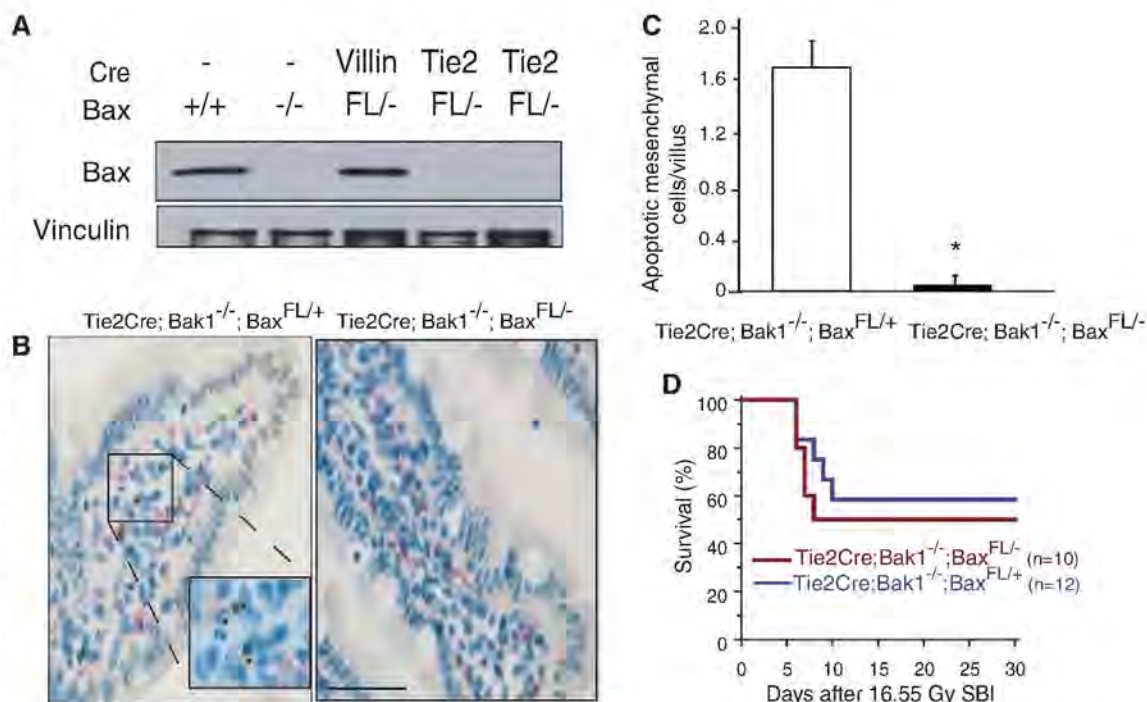
At radiation doses that can cause the GI syndrome, mitotic death occurs within the small intestine (17). Although the molecular mecha-

nism of this type of cell death is not completely understood, it may involve progression through an aberrant mitosis with damaged DNA (28). Therefore, we looked for evidence of aberrant mitosis in the crypts of the small intestine after irradiation. In un-irradiated mice, almost all cells undergoing mitosis showed chromosomes that aligned symmetrically (Fig. 4A and fig. S6). Twenty-four hours after 13.4 Gy of SBI, most of the mitotic cells in the crypts were aberrant, because chromosomes aligned in an asymmetrical fashion with lagging chromosomes or in micronuclei (Fig. 4A and fig. S6). Because p53 is required in the small intestine for optimal cell cycle arrest after radiation to prevent inappropriate entry into mitosis (19), we generated mice in which p53 was deleted in the GI epithelium (*VillinCre; p53<sup>FL/+</sup>*) to investigate whether cell cycle checkpoints in GI epithelial cells regulate the GI syndrome. Indeed, the *VillinCre; p53<sup>FL/+</sup>* mice were more sensitive to

**Fig. 1.** Deletion of the intrinsic pathway of apoptosis in the hematopoietic system protects mice from death after WBI. (A) Western blot for BAX and tubulin in spleen, lymph node, and thymus from *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice (FL/-) demonstrates deletion of *Bax* in the hematopoietic system. *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice (FL/+) retain *Bax* and serve as a control. (B) Kaplan-Meier survival analysis of *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice and *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* littermates after 12.5 Gy of WBI. By log-rank comparison,  $P = 0.0001$ .



**Fig. 2.** Deletion of the intrinsic pathway of apoptosis from endothelial cells does not protect mice from SBI. (A) Western blot for BAX and vinculin from lung endothelial cells isolated from mice that contained no Cre (-), *VillinCre* (*Villin*), or *Tie2Cre* (*Tie2*) and the indicated *Bax* genotype. (B) Mesenchymal cell (endothelial cell and lymphocyte) apoptosis is decreased in *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice. TUNEL (brown) and MECA-32 (pink) immunohistochemistry identify apoptotic mesenchymal cells within the villi of *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice. Scale bar, 100  $\mu$ m. (C) Quantification of mesenchymal cell apoptosis in the villi of the distal small intestine. The mean number of mesenchymal cells undergoing apoptosis in a total of at least 87 villi from three mice is shown; \* $P < 0.01$  by two-tailed  $t$  test. Error bars indicate SEM. (D) Kaplan-Meier survival analysis of *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/+</sup>* mice and *Tie2Cre; Bak1<sup>-/-</sup>; Bax<sup>FL/-</sup>* littermates after 16.55 Gy of SBI. By log-rank comparison,  $P =$  not significant (NS).





SBI than *VillinCre*; *p53<sup>FL/+</sup>* littermates (Fig. 4B), even though we did not observe a difference in surviving crypts at 90 hours after SBI (fig. S7). These results point to cells within the GI crypt epithelium as the primary cellular target of radiation mediating the GI syndrome. Moreover, as reported by others (15), we also found mice lacking the cyclin-dependent kinase inhibitor p21 to be sensitive to the GI syndrome (fig. S8). To investigate whether deletion of the intrinsic pathway of apoptosis could suppress the sensitivity of the GI syndrome that results from loss of p53, we generated mice (*VillinCre*; *p53<sup>FL/-</sup>*; *Bak1<sup>-/-</sup>*; *Bax<sup>FL/-</sup>*) in which p53 and the intrinsic pathway of apoptosis were deleted

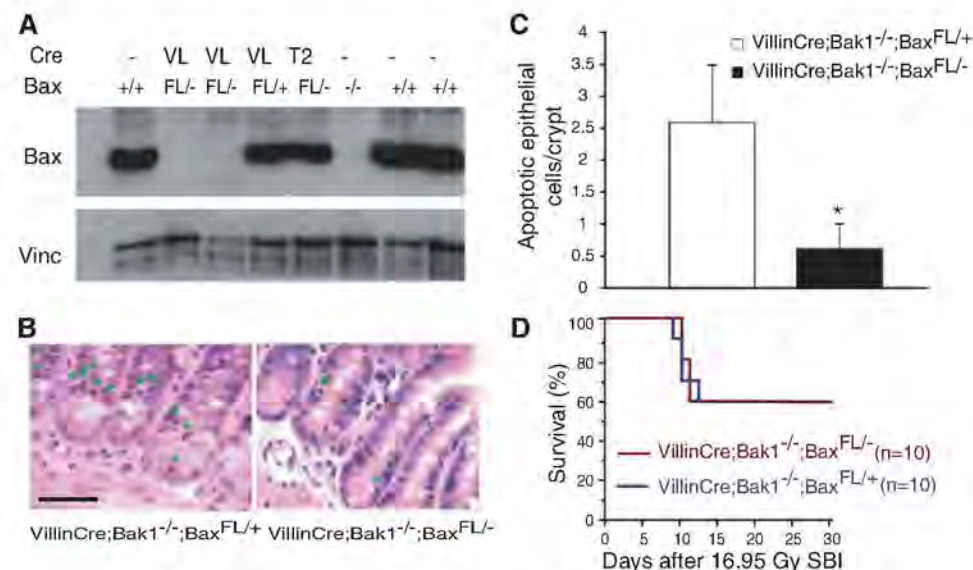
from GI epithelial cells (25). Despite deletion of *Bak1* and *Bax*, mice remained sensitive to the GI syndrome when p53 was deleted in GI epithelial cells (Fig. 4C).

These results are consistent with a model in which progression through p53-regulated cell cycle checkpoints after radiation leads to the re-productive or mitotic death of GI epithelial cells independent of apoptosis to cause the GI syndrome (16, 17, 19). To test this model in a gain-of-function experiment, we studied "super-p53" *tg<sup>b</sup>* mice, which contain two extra copies of the wild-type gene encoding p53 (29). After irradiation, super-p53 mice exhibit an enhanced DNA damage response with elevated levels of

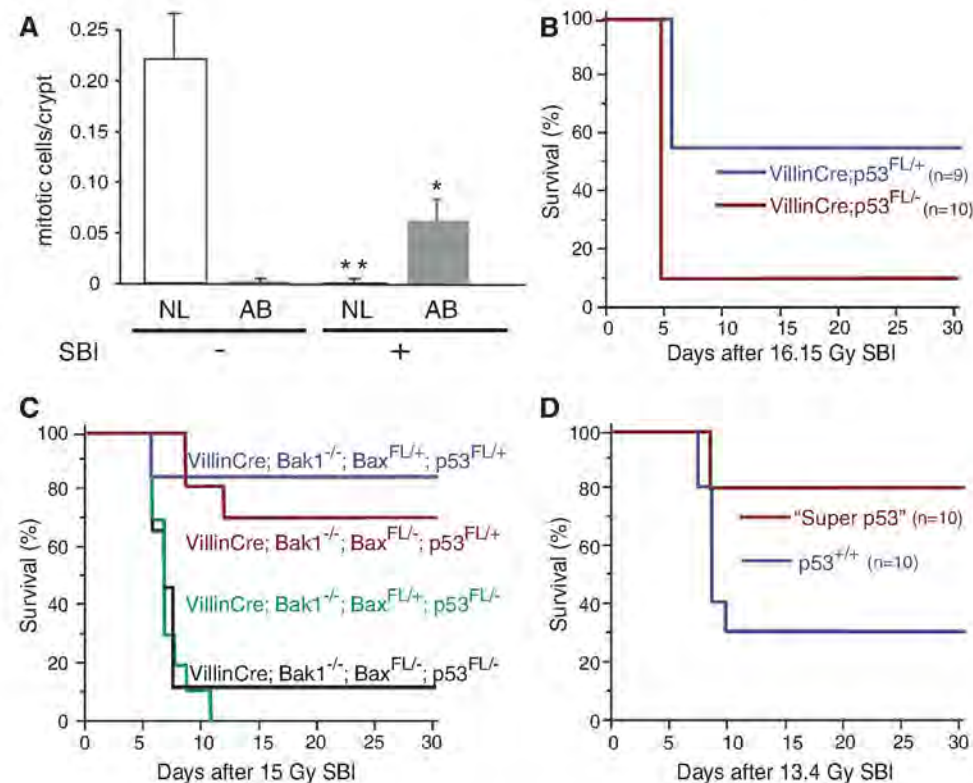
p53-dependent apoptosis and increased induction of the cyclin-dependent kinase inhibitor p21 (29). Consistent with a model in which p53-dependent cell cycle arrest regulates the GI syndrome, we observed that p53 *tg<sup>b</sup>* mice are protected from the GI syndrome after SBI (Fig. 4D).

We also generated mice in which p53 was deleted from endothelial cells. These *Tie2Cre*; *p53<sup>FL/-</sup>* mice were not more sensitive to the acute GI syndrome after SBI (fig. S9). Surprisingly, all of these mice died from delayed radiation effects within 2 months (fig. S9). Therefore, p53 may not only protect the GI epithelium from acute injury (15) but may also protect endothelial cells from late effects from radiation.

**Fig. 3.** Deletion of the intrinsic pathway of apoptosis from GI epithelial cells does not protect mice from SBI. (A) Western blot for BAX and vinculin from GI epithelial cells isolated from mice without Cre (–), with VillinCre (VL), or Tie2Cre (T2), and the indicated *Bax* genotype. (B) Hematoxylin and eosin–stained sections of the distal small intestine 4 hours after 16.95 Gy of SBI demonstrate suppression of apoptosis within the crypt epithelium in *VillinCre*; *Bak1<sup>-/-</sup>*; *Bax<sup>FL/-</sup>* mice. A green asterisk labels cells with characteristic apoptotic morphology. Scale bar, 100  $\mu$ m. (C) Quantification of GI epithelial cell apoptosis in the crypts of the distal small intestine. The mean number of apoptotic cells in a total of at least 100 crypts in three mice is shown. Error bars indicate SEM. \* $P < 0.01$ , by two-tailed *t* test. Similar results were observed in the proximal small intestine. (D) Kaplan-Meier survival analysis of *VillinCre*; *Bak1<sup>-/-</sup>*; *Bax<sup>FL/+</sup>* and *VillinCre*; *Bak1<sup>-/-</sup>*; *Bax<sup>FL/-</sup>* mice after 16.95 Gy of SBI. By log-rank comparison,  $P = NS$ .



**Fig. 4.** Regulation of the GI syndrome by p53 in epithelial cells is independent of apoptosis. (A) Quantification of normal (NL) and aberrant (AB) mitoses in GI crypt epithelial cells of the proximal and distal small intestine from wild-type mice without (–) or 24 hours after (+) 13.4 Gy of SBI with ionizing radiation. The mean number of mitoses per crypt in a total of at least 115 crypts from six mice is shown. Error bars indicate SEM. \* $P < 0.04$  for aberrant mitoses, two-sided *t* test; and \*\* $P < 0.005$  for normal mitoses, two-sided *t* test. (B) Kaplan-Meier survival analysis of *VillinCre*; *p53<sup>FL/+</sup>* and *VillinCre*; *p53<sup>FL/-</sup>* mice after 16.15 Gy of SBI with gamma irradiation.  $P = 0.0015$  by log-rank test. (C) Kaplan-Meier survival analysis of at least nine mice of each of the indicated genotypes after 15 Gy of SBI with gamma irradiation.  $P = 0.0001$  between *p53<sup>FL/-</sup>* and *p53<sup>FL/+</sup>* mice and  $P = NS$  between *Bax<sup>FL/-</sup>* and *Bax<sup>FL/+</sup>* mice by log-rank test. (D) Kaplan-Meier survival analysis of super-p53 *tg<sup>b</sup>* mice and C57/BL6 *p53<sup>+/+</sup>* littermate controls after 13.4 Gy of SBI with ionizing radiation.  $P = 0.02$  by log-rank test.





These results have potential implications for efforts to develop medical countermeasures against radiation. Drugs that block the intrinsic pathway of apoptosis may be effective for preventing the hematopoietic syndrome but not the GI syndrome. Indeed, the p53 inhibitor pifithrin- $\alpha$  rescues mice from the lethal effects of the hematopoietic syndrome but does not protect mice from the GI syndrome (15). Because complete inhibition of p53 may promote the GI syndrome (Fig. 4B), drugs to treat the acute radiation syndrome that block apoptosis should not compromise p53 function (30).

This study may also have important implications for cancer therapy. Inhibitors of p53 function have been proposed as a way to limit the acute toxicity of radiation therapy and increase tumor response without increasing the risk of radiation-induced cancers (31–33). For many patients, acute toxicity is not dose-limiting in the clinic. Instead, the risk of late effects from radiation therapy, which can be secondary to vascular injury (34), frequently limits the radiation dose that can safely be delivered to treat a cancer. Our finding that deleting p53 from endothelial cells sensitizes mice to late effects of radiation (fig. S9) raises the possibility of increased late effects when p53 inhibitors are combined with radiation therapy.

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Materials and Methods

SOM Text

Figs. S1 to S9

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**POSTDOCTORAL FELLOW POSITION**, University of Alabama at Birmingham, U.S.A., to study molecular mechanisms in kidney disease (*J. Biol. Chem.* 281:39681-39692, 2006; *J. Clin. Invest.* 112:209-221, 2003) and novel glomerular therapeutics (in revision in leading journal). Ph.D. in cell and molecular biology required. Experience in kidney disease is not necessary. Electronically send curriculum vitae to **Sumant S. Chugh**, M.D., e-mail: [chugh@uab.edu](mailto:chugh@uab.edu).

## POSITIONS OPEN

**UND THE UNIVERSITY OF  
NORTH DAKOTA**

### GRASSLAND ECOLOGY

We seek to fill a **TENURE-TRACK POSITION** with open rank. The position is integral to our undergraduate major in Fisheries and Wildlife Biology and will also strengthen the Department of Biology's teaching and research mission in basic science. The individual will combine hypothesis-driven research on community or ecosystem ecology of grasslands, with the ability to interact with resource management and conservation agencies. Teaching duties will not exceed two courses per year during the first several years. Courses taught will depend on the candidate's area of expertise and may include participation in a team-taught introductory biology course. The successful candidate will demonstrate the ability to establish a productive and extramurally funded research program and actively train M.S. and Ph.D. students. Position will begin 16 August 2010; Ph.D. is required, with postdoctoral experience desirable. Review of applications will begin February 12, 2010, and continue until the position is filled. Send curriculum vitae, three representative reprints, statement of teaching and research interests, and names and contact information for three references to: **Dr. Robert Newman** (e-mail: [robert.newman@und.edu](mailto:robert.newman@und.edu)), 10 Cornell Street Stop 9019, University of North Dakota, Grand Forks, ND 58202-9019. For more information, see website: <http://www.und.edu/dept/biology/jobs.htm>.

The University of North Dakota is an Equal Opportunity/Affirmative Action Employer, and we strongly encourage applications from women and underrepresented groups.

### ASSISTANT PROFESSOR, MARINE ICHTHYOLOGIST/FISH BIOLOGIST Moss Landing Marine Laboratories and San José State University

Moss Landing Marine Laboratories and San José State University invite applications for a tenure-track Assistant Professor position beginning 23 August 2010. We are seeking a marine scientist with broad interests in fish biology with an emphasis on the ecology of marine fishes. The successful applicant must have a strong commitment to quality instruction and will be expected to pursue a vigorous research program involving Master's of Science (M.S.) students. A Ph.D. is required. Instructional duties will include teaching ichthyology, marine ecology (team taught), population biology, and other courses in the applicant's area of expertise. We also expect the successful candidate will develop an active research program using extramural funding to support their own research and that of their graduate students. Applicant should have an awareness of and sensitivity to the educational goals of a multicultural population.

MLML, located in a new laboratory on Monterey Bay, is operated by a consortium of California State University campuses (Fresno, Hayward, Monterey Bay, Sacramento, San Francisco, San José, and Stanislaus). MLML offers undergraduate courses but is primarily a graduate institution for Consortia students seeking a Master of Science degree in marine science. The faculty appointment is through San José State University, but the successful candidate will be in residence at Moss Landing Marine Laboratories. For further information about MLML, visit website: <http://www.mlml.calstate.edu>.

Application should include a cover letter, curriculum vitae, statement of teaching interests/philosophy, statement of research interests, and names and contacts for three references. For full consideration, all materials should be submitted by March 15, 2010, to: **Dr. James T. Harvey**, Chair of Ichthyology Search Committee, Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039. Please include Job Opening ID (JOID: 13750) on all correspondence.

SJSU is an Equal Opportunity/Affirmative Action Employer committed to the core values of inclusion, civility, and respect for each individual.





**Department of Health and Human Services**

**National Institutes of Health**

**Deputy Director of NIH**

**Division of Program Coordination, Planning, and Strategic Initiatives**



The Office of the Director (OD), National Institutes of Health (NIH) in Bethesda, Maryland, is seeking a Director of the newly created Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI). Exceptional candidates with the scientific vision to identify innovative, high impact science and the ability to integrate research across traditional disciplines are encouraged to apply.

The DPCPSI Director will serve as a Deputy Director of the NIH and report to the NIH Director. The primary responsibilities of the Division are to (1) develop innovative, high-risk high-reward initiatives that will have national and international impact, supported by the NIH Common Fund; (2) advise the NIH Director on issues involving trans-NIH planning, analysis, implementation, performance assessment, and evaluation activities; (3) develop and conduct scientific analyses relevant to NIH research portfolio analysis; and (4) coordinate the activities of the DPCPSI Program Offices (Office of AIDS Research; Office of Research on Women's Health; Office of Behavioral and Social Sciences Research; Office of Disease Prevention; and Office of Strategic Coordination) to maximize their collective impact and to ensure that their efforts are aligned with the mission of NIH.

The DPCPSI Director will exercise leadership, initiative, and creativity in establishing and maintaining relationships with key Federal and non-Federal officials, nationally and internationally recognized scientific leaders and officials of academic, research, and other institutes and organizations, and professional and advocacy groups.

Salary is commensurate with experience; a full package of benefits (including retirement, health, life, long term care insurance, Thrift Savings Plan participation, etc.) is available.

A Search Committee chaired by Drs. Katherine Hudson and Lawrence Tabak will review applications for this position. A detailed vacancy announcement that includes mandatory qualifications requirements, and application procedures may be obtained at NIH's Executive Jobs site: <http://www.jobs.nih.gov/vacancies/executive.htm>, or by calling Regina Reiter at (301) 402-1130. Interested applicants must send a Curriculum Vitae, Bibliography, a Vision Statement, and responses to the qualifications requirements, *electronically*, to Ms. Regina Reiter, at [SeniorRe@OD.NIH.GOV](mailto:SeniorRe@OD.NIH.GOV), (301) 402-1130. If you need additional information, please contact Ms. Reiter at 301-402-1130.

**Applications must be received by close of business March 8, 2010.**

*DHHS and NIH are Equal Opportunity Employers*



**DHHS, National Institutes of Health  
 National Library of Medicine  
 Staff Scientist position**

The National Center for Biotechnology Information (NCBI) at the National Institutes of Health (NIH), located in Bethesda, MD, performs research in computational biology and creates and maintains information systems and computational tools for the biological research community. The Information Engineering Branch is seeking a Staff Scientist to join the dbVar team.

dbVar is a database of genomic structural variants developed at NCBI. The identification of large-scale structural variants and the understanding of how these variants contribute to phenotypes is a rapidly growing field and critical to our understanding of human health and disease. The responsibilities of the Staff Scientist will include research and development, data curation and review and data management. The successful candidate needs to be able to perform whole genome analysis as well as curate specific regions.

We are looking for an individual with a track record of outstanding research in population genetics, high-throughput sequencing, comparative genomics, array based technologies, next generation sequencing and/or computational biology. Candidates may be U.S. or non-U.S. citizens and must have a Ph.D., strong publication record, and postdoctoral experience. The successful candidate will have excellent communication skills and proven ability to successfully engage into a multi-disciplinary collaborative research. Successful candidates will serve on a non-competitive appointment in the excepted service. Salary is competitive, commensurate with education and experience. A full package of benefits is available.

Interested individuals should send a copy of their CV, cover letter detailing research interests, and names of three references to Deanna Church ([church@ncbi.nlm.nih.gov](mailto:church@ncbi.nlm.nih.gov)).

Applications will be accepted through **May 31, 2010**.

For additional information on the National Center for Biotechnology Information, please see our website at: <http://www.ncbi.nlm.nih.gov/>.

**The NIH Director's  
 Wednesday Afternoon Lecture Series**

Biomedical scientists around the world are invited to join us online to hear leading investigators present their latest results to the NIH Intramural Research community. Lectures may be viewed live at 3:00 p.m., EST (20:00 GMT) on Wednesdays, from September through June. Live webcasts can be viewed under "Today's Events" at: <http://videocast.nih.gov/>

The current schedule of lectures is available at: <http://wals.od.nih.gov>

**Upcoming Lectures:**

**February 3: Ellen Rothenberg, California Institute of Technology "Stem Cell to T Cell: Molecular Anatomy of Commitment"**

**February 10: Fred Gage, The Salk Institute for Biological Studies, "Neural Plasticity and Diversity in the Adult Mammalian Brain"**

**February 17: Helen Mayberg, Emory University, "Turning Depression Circuits Using Deep Brain Stimulation"**

**February 24: Michael Dustin, New York University, "Creating Super-regulatory T Lymphocytes"**



## Lawrence Berkeley National Laboratory



### Environmental Energy Technologies Division Director

Lawrence Berkeley National Laboratory is looking for a proven leader to be its next Division Director for the Environmental Energy Technologies Division (EETD) at an exciting time of increased national and global attention on research, development, analysis and technical assistance to address energy and climate challenges. The Division has a multidisciplinary staff of approximately 450 scientists, engineers, technical and administrative personnel, faculty and students, and an annual budget of over \$60M. EETD research is primarily funded by the U.S. Department of Energy's (DOE) Office of Energy Efficiency and Renewable Energy, with significant support from other DOE offices and federal agencies, and the State of California. The Division performs research on advanced energy technologies, energy efficiency, indoor environmental quality, atmospheric science, and climate change. The Division has a substantial effort in energy analysis, with a primary emphasis on energy demand and efficiency. EETD research is also coupled to Laboratory-wide initiatives on new and secure energy sources for the U.S. For more information about EETD and its scientific programs, visit the website at <http://eetd.lbl.gov>.

In the world of science, Lawrence Berkeley National Laboratory (Berkeley Lab) is synonymous with excellence. Berkeley Lab is an incubator for ideas, innovations and products that help society and explain how the Universe works. Located on a 200 acre site in the hills above the University of California's Berkeley campus, adjacent to the San Francisco Bay, the Lab is managed by the University of California, has an annual budget of more than \$650 million and a staff of about 3,900 employees. As a University of California employee, you will enjoy a generous benefits package. For more information about the Lab go to <http://www.lbl.gov>.

The successful candidate will provide scientific leadership for the division's research programs; develop new programs that address the national needs in energy and environmental areas; and act as chief spokesperson for the division in interactions with external agencies, including the Department of Energy. The Director will collaborate with other Division Directors and build collaborative programs with UC Berkeley and other research institutions. The Director is responsible for all areas of management to ensure maximum effectiveness within the division, which include operations, environmental health and safety, administration, budget, and human resources.

Candidates must have a distinguished record of scientific accomplishments in a discipline relevant to the research of the Environmental Energy Technologies Division; experience with scientific management and development; demonstrated ability to interact effectively with funding agencies such as the Department of Energy; and must be capable of providing scientific leadership for a multidisciplinary group of scientists. A Ph.D. in a scientific discipline relevant to the Environmental Energy Technologies Division is preferred.

#### How to Apply

For a complete position description and to apply directly online, please visit <http://cjo.lbl.gov>, select "Search Jobs", and enter 23715 in the keyword search. Please follow the online instructions to complete the application process.

As part of the online application process, please submit a single attachment that includes both your resume or CV and a statement of your research interests. Please be sure to reference where you found out about the position.



LBNL is an Equal Opportunity/  
Affirmative Action Employer.



UMASS  
AMHERST

### Assistant Professor

#### Department of Plant, Soil, and Insect Sciences

The Department of Plant, Soil and Insect Sciences, University of Massachusetts Amherst, invites applications for an Assistant Professor level, tenure-track position in Molecular Biology or related area beginning Fall 2010. Successful candidates will have a Ph.D., a strong record of research, including clear potential to obtain extramural research support and maintain an active research program. In addition, it is anticipated that the incumbent will participate in teaching an expanding curriculum in Biotechnology in our Department. Candidates with a research focus in Metabolomics/Genomics and/or Biotic Stress/ Crop Protection are especially encouraged to apply. We have special interests in recruiting faculty to our growing group of molecular biologists in the Department. The position is one of several on campus designed to expand an existing cluster focused on biofuels and renewable energy research.

Applicants should send a vita, statements of research and teaching interests, reprints of recent publications, and arrange for at least three letters of recommendation to: Dr. Anne Averill, Search Committee Chair, Department of PSIS, Fernald Hall, University of Massachusetts, Amherst, MA 01003-7710. The search committee will begin reviewing applications on 1 February and will continue until the position is filled. Hiring is contingent upon the availability of funds.

*The University provides an intellectual environment committed to providing academic excellence and diversity including mentoring programs for faculty. The College and the Department are committed to increasing the diversity of the faculty, student body and the curriculum. The University of Massachusetts is an Affirmative Action/ Equal Opportunity Employer. Women and members of minority groups are encouraged to apply.*

### Associate Professor Genitourinary Section, Department of Medicine

The Genitourinary Section, Department of Medicine at the Roswell Park Cancer Institute invites applications for an Associate Professor level position. Outstanding candidates with demonstrated excellence in basic research in genitourinary malignancies and peer-reviewed funding in tumor angiogenesis and/or renal cell carcinoma will have high priority. Responsibilities will include conducting basic laboratory research in collaboration with translational and clinical researchers of the Genitourinary Program and other programs at RPCI. The Roswell Park Cancer Institute is committed to increasing representation of women and members of minority groups in our faculty and encourages applications from such candidates. Applicants must hold a Ph.D. and/or M.D. degree.

Interested individuals should forward a C.V. and letter describing interests to Roberto Pili MD, Chief of the Genitourinary Section, RPCI, [Roberto.Pili@RoswellPark.org](mailto:Roberto.Pili@RoswellPark.org); or Alex Adjei MD PhD, Chair of the Department of Medicine, RPCI, [Alex.Adjei@RoswellPark.org](mailto:Alex.Adjei@RoswellPark.org) or fax a copy to 716-845-8232.

Roswell Park Cancer  
Institute is an Affirmative  
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ROSSELL  
PARK  
CANCER INSTITUTE





The University of Texas Medical Branch

**FACULTY POSITIONS**  
**Center for Biodefense and Emerging Infectious**  
**Diseases, Cancer Center, and the**  
**Department of Pathology**

The University of Texas Medical Branch (UTMB) has Assistant, Associate or Full professor level, tenure-track positions available for persons with MD, PhD, and/or DVM degrees. UTMB has a very rich environment for infectious diseases research, and is home to the Galveston National Laboratory ([www.utmb.edu/gnl](http://www.utmb.edu/gnl)), the Center for Biodefense and Emerging Infectious Diseases ([www.utmb.edu/CBEID/links.shtml](http://www.utmb.edu/CBEID/links.shtml)), the Cancer Center (<http://www.utmb.edu/cancer/>), the Center for Hepatitis Research ([www.utmb.edu/chr](http://www.utmb.edu/chr)), the Institute for Human Infections and Immunity ([www.utmb.edu/ihii/index.html](http://www.utmb.edu/ihii/index.html)), the Institute for Translational Science ([www.its.utmb.edu](http://www.its.utmb.edu)), and the Sealy Center for Structural Biology and Molecular Biophysics ([www.scsb.utmb.edu](http://www.scsb.utmb.edu)). This wealth of expertise and state-of-the-art high containment BSL3 and BSL4 and core facilities offer outstanding opportunities for collaboration and multidisciplinary research with over 150 scientists focusing on infectious disease/immunology and cancer research.

**Assistant or Associate Professor in the Neuropathogenesis of Infectious Diseases:** The Center for Biodefense and Emerging Infectious Diseases and the Department of Pathology are seeking applications from candidates with a desire to pursue a career in the neuropathogenesis of infectious diseases, particularly acute viral or bacterial infections that affect the central nervous system. The ideal candidate will have a strong background in neuroscience, and it is expected that future research will include biohazardous infectious agents. Outstanding collaborative opportunities are available on neuropathogenic agents. The successful candidate will develop an independent program of externally funded research. Particularly attractive opportunities exist to build collaborative projects in vaccine development, endothelial cell pathobiology, and/or animal models of infectious diseases. In addition to a start-up package and laboratory space, UTMB offers competitive internal grants for junior faculty.

**Associate or Full Professor in Cancer Research:** The Cancer Center is seeking applications from candidates with proven abilities in research relating to cancer pathogenesis. The successful applicant will have outstanding opportunities to play a major leadership role in the expansion of UTMB's cancer research programs. UTMB has an NIH-sponsored Clinical and Translational Science Award, in addition to being home to an Institute for Translational Sciences. These programs provide additional opportunities and facilities for basic, translational and clinical cancer research. The recently established Cancer Prevention and Research Institute of Texas also offers exceptional funding opportunities for Texas researchers, with \$450M in state funds committed over the next two years.

**Faculty for the Endothelial Pathobiology Program:** The Department of Pathology is seeking applications for faculty members to participate in the developing Endothelial Pathobiology Program. Members of the Program will interact with University scientists whose research is focused on infectious, immunological, metabolic, nutritional, toxicological, gestational, or endocrine diseases in which the endothelium of the microvasculature plays a key role in pathogenesis. The successful applicant will have an active research project(s) in the field of endothelial cell pathobiology which employs cutting-edge technologies and physiologically relevant models of microvasculature dysfunction. Developing collaborative research initiatives is essential, as is familiarity with research-based postgraduate and student research programs.

Interested candidates should send a CV, outline of research interests, and names of four references electronically to **Dr. Kimberly Schuenke, CBEID Associate Director** ([kischuen@utmb.edu](mailto:kischuen@utmb.edu)), and specify the position of interest. For additional information, contact Dr. Schuenke by email or phone at **409-747-0766**.

*The University of Texas Medical Branch at Galveston is an Equal Opportunity, Affirmative Action Institution which proudly values diversity. Candidates of all backgrounds are encouraged to apply.*



WORKING AT  
 THE UNIVERSITY OF GENEVA

The **FACULTY OF MEDICINE** and the **UNIVERSITY HOSPITALS** of GENEVA are seeking a :

**FULL or ASSOCIATE PROFESSOR OF NEUROLOGY and**  
**HEAD OF THE DIVISION OF NEUROLOGY**

within the academic department of clinical neuroscience of the Faculty of medicine of the University of Geneva and within the clinical department of clinical neuroscience of the Geneva University Hospitals

We are seeking a specialist for a university hospital position that holds a professorship component (full 4/10th or associate 3/10th professor) as well as Head of the Division of neurology component (10/10th).

Candidates must have a large experience in clinical neurology, a broad view of the speciality, and be able to integrate and promote the various sub-specialities of neurology.

The candidates are able to ensure high quality teaching in neurology on the graduate, postgraduate and CME level and are willing to supervise and promote both clinical and academic careers in the field.

In addition to the clinical and teaching qualities, candidates must have a high-level international scientific record in a field of neurology. Ideally, their research integrates clinical and basic research. Given the current expertise of the Division in cognitive neurosciences, a research in this domain would be an asset.

The candidate must have a broad vision of the field that will enable him/her to manage a division, which encompasses a variety of perspectives - each one related to a specific field of clinical expertise - within an environment characterised by multiple interests.

The following criteria will also be taken into consideration :

1. Capacity to federate specialized domains in the field of neurology within a single clinical and academic structure.
2. Aptitude to interact harmoniously with the different departments of clinical neuroscience, with the department of fundamental research as well as the Geneva Neuroscience Center.

**REQUIRED TITLE:** Physician diploma of specialisation in neurology (FMH title or equivalent).

**START OF TERM:** 1st October 2010 or date to be discussed

Applications must be sent before **March 15th 2010** to:

Professor Jean-Louis CARPENTIER, Dean  
 Faculty of medicine, University of Geneva  
 Office of the Dean /CMU  
 1, rue Michel-Servet  
 CH-1211 Geneva 4

Further particulars regarding job description, terms of recruitment as well as application directives may be obtained from Mrs Sylvia de Raemy, [sylvia.deraemy@unige.ch](mailto:sylvia.deraemy@unige.ch)  
 Tel: +41 22 379 50 26

*In order to promote gender equality, the University of Geneva encourages women to apply.*





# Chief Research Officer, The Children's Hospital and University of Colorado Denver School of Medicine, Aurora, Colorado

This is a senior management executive position at The Children's Hospital overseeing The Children's Hospital Research Institute. Concurrently, the Chief Research Officer (CRO) will hold the position of Associate Dean for Child Health Research at University of Colorado Denver (UCD). The CRO will be responsible for development and implementation of the strategic direction and agenda for research programs at The Children's Hospital. The successful candidate will have exceptional clinical and/or laboratory research achievement at the national and international levels; demonstrated leadership and administrative skills in the advancement of research efforts; demonstrated ability to develop and implement a strategic research vision; excellent interpersonal and organizational skills; experience in institutional and academic collaboration; and the ability to lead diverse groups within an academic and healthcare setting.

Review of applications will begin January 1, 2010 and continue until the position is filled.

For more information and for application procedure: [www.ucdenver.edu](http://www.ucdenver.edu)

Click Employment > Click Search Postings. Keyword search: Chief Research Officer

Search Committee Chairman: Philip Zeitler MD, PhD, [zeitler.philip@tchden.org](mailto:zeitler.philip@tchden.org)

Salary is commensurate with skills and experience. Information about University of Colorado benefits is found at [www.cusys.edu/pbs](http://www.cusys.edu/pbs). UCD is dedicated to ensuring a safe and secure environment for our faculty, staff, students and visitors. To achieve that goal, we conduct background investigations for all prospective employees. The University of Colorado is committed to diversity and equality in education and employment.

University of  
Colorado Denver  
School of Medicine



The Children's Hospital

## GLADSTONE INSTITUTE OF NEUROLOGICAL DISEASE

### Assistant Professor/Assistant Investigator Neurobiology of Disease Gladstone/UCSF Faculty Position

The Gladstone Institute of Neurological Disease and the University of California, San Francisco (UCSF) invite applications for a faculty position at the level of Assistant Professor/Assistant Investigator. Primary criteria for appointment will be genuine commitment to disease-related neuroscience and outstanding records of innovative research and academic performance. Of particular interest are candidates who are seeking their first independent faculty position, focus on Alzheimer's disease, and have expertise in the pathobiology of mitochondria, stem cells or systems biology. A strong publication record and clear potential to establish a vigorous independent research program are essential.

The successful candidate will join an interactive group of investigators in Gladstone's state-of-the-art research facility at UCSF's Mission Bay campus. S/he will have joint appointments in the Gladstone Institute of Neurological Disease and in the Department of Neurology, the Neuroscience Graduate Training Program and the Biomedical Sciences Graduate Training Program at UCSF. Excellent salary support is provided. Women and underrepresented minorities are encouraged to apply. Please send curriculum vitae, description of achievements and research interests, and the names of three references to:

[gindsearch@gladstone.ucsf.edu](mailto:gindsearch@gladstone.ucsf.edu)  
GIND/UCSF Search Committee  
1650 Owens Street  
San Francisco, CA 94158

*The J. David Gladstone Institutes and UCSF are Affirmative Action/Equal Opportunity Employers. They undertake affirmative action to assure equal employment opportunity for underutilized minorities and women, for persons with disabilities, and for Vietnam-era veterans and special disabled veterans. Gladstone and UCSF seek candidates whose experience, teaching, research or community service has prepared them to contribute to our commitment to diversity and excellence.*

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## personalized medicine in the clinic:

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This national conference with top experts will examine the impact of personalized medicine on the delivery of healthcare in the future. Conference highlights:

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ethics  
individualized medical care  
economics  
liability issues for physicians

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## National Institute of Mental Health Division of Intramural Research Programs Tenure Track Investigator

The Division of Intramural Research Programs (DIRP), National Institute of Mental Health (NIMH), National Institutes of Health (NIH), seeks a highly accomplished Tenure Track Investigator to develop an independent research program in statistical genetics. (Applications from tenured investigators will be considered.) The candidate will have the opportunity for collaboration with scientists in diverse research areas in genetics (e.g., genetic epidemiology, biostatistics, statistical genetics, molecular genetics), as well as neuroscience and relevant clinical disciplines.

The position comes with a budget and staff. The strong scientific environment and outstanding resources at NIMH make this a unique opportunity for a high-achieving scientist. The position also offers unparalleled opportunities for interdisciplinary collaborations with scientists throughout the NIH. The successful candidate will be expected to strengthen the current program by establishing a program in statistical genetics that applies and develops statistical methods to analyze the role of genes in the etiology of complex disorders, particularly neuropsychiatric disorders.

Applicants should have a Ph.D. in: statistics, population genetics, mathematics, genetic epidemiology, or related fields. The applicant should have experience and interest in statistical analysis of human genetic data, and in developing new methods or adapting traditional statistical methods to identify the role of genes in the etiology of complex disorders.

Salary is commensurate with experience and accomplishments, and a full Civil Service package of benefits (including retirement, health, life, and long-term care insurance, as well as a Thrift Savings Plan, etc.) is available. NIMH is a major research component of the National Institutes of Health and the Department of Health and Human Services, which have nationwide responsibility for improving the health and well-being of all Americans. Interested applicants should send curriculum vitae, bibliography, statement of research interests, accomplishments, and goals, together with 3 letters of reference to: Chair, Search Committee for Statistical Geneticist, NIMH, NIH, Bldg. 10, Rm. 4N-222, 9000 Rockville Pike, Bethesda, MD 20892; or e-mail to: [steyerm@mail.nih.gov](mailto:steyerm@mail.nih.gov). Review of applications will begin **March 31, 2010**, but applications will continue to be accepted and considered until the position is filled.



**NIH and DHHS are Equal Opportunity Employers**



### Post-doctoral Position Available Immediately

Dana-Farber Cancer Institute, Department of Cancer Immunology and AIDS, is seeking a post-doctoral fellow with expertise in molecular virology to join a dynamic team involved in studying lentiviral pathogenesis and host immunity in primate models. The project involves construction of mutant proviruses and the analysis of their biology and evolution in primates. The position offers training in primate lentivirology and immunology. Ph.D. in molecular biology/virology is required; a strong background in gene expression analysis is a plus. The successful candidate will have a joint appointment at Harvard Medical School and the Dana-Farber Cancer Institute.

Interested candidates should send their CV and the names of three references by e-mail to:

**Dr. Ruth Ruprecht**  
Dana-Farber Cancer Institute  
44 Binney Street  
Boston, MA 02115  
e-mail:  
[ruprecht\\_lab@dfci.harvard.edu](mailto:ruprecht_lab@dfci.harvard.edu)

*Invest your career where you can  
make a Difference!*

The Duke Human Vaccine Institute, occupying a place of national and international leadership in the fight against major infectious diseases, is currently recruiting for Postdoctoral positions.

### Molecular Virology & Immunology

This position will study the impact of genetic variation of HIV-1 on viral biological phenotypes and vaccine development. Highly motivated individuals with a recent Ph.D. and/or M.D. and experience in RT-PCR, cloning, sequence analysis, virus culture and neutralization assays are encouraged to apply.

### B Cell Immunoregulation

This position will study the role of B cells in the immune response to HIV. The project will specifically involve characterizing B cell immune modulation mechanisms in a variety of knock-in mouse models and using these models to test if/what immunization regimens can elicit the production of broadly neutralizing antibodies. Qualified candidates should have experience in molecular and cellular immunology techniques and extensive experience using flow cytometry. Preference will be given to candidates with experience in characterizing mouse knock-out/knock-in models and performing mouse breedings and immunizations. A solid background in B cell immunology is also a plus.

Duke University Medical Center is located in the energetic and progressive Research Triangle area of North Carolina and was named by the *Chronicle of Higher Education* as one of the "2008 Great Colleges to Work For." Duke is also ranked among the top 35 institutions for best places to work for Postdocs (*The Scientist*).

**Qualified candidates should send a cover letter, current CV, description of recent research work, and references to:**

**Duke Human Vaccine Institute**  
Box 103020, 106 Research Drive, Durham, NC 27710  
Email: [dhvi.careers@notes.duke.edu](mailto:dhvi.careers@notes.duke.edu)

Please note for which position you are applying in the subject line.



**Duke Human Vaccine Institute**

Duke University Medical Center is an equal opportunity/affirmative action employer.





We strive to deliver the best – in patient care, research, and education. Experience amazing opportunities and outstanding rewards with the University of Miami, Miller School of Medicine.

### ASSOCIATE SCIENTIST

The Department of Ophthalmology has an immediate opening for an Associate Scientist. The Associate Scientist will work directly with the Principal Investigator; will also plan and execute laboratory research project in close collaboration with the Lab Director. Duties will include the supervision of junior trainees such as students; keep dated, detailed, well-organized and up-to-date lab notebook which serves as a record of the rationale, the experimental design, the materials and procedures used, any changes to the work as performed, and the results, including all documentation. S/he will participate in lab meetings, giving presentations as assigned. Participation in all departmental research activities including journal clubs, lab conference & seminars; preparation of manuscripts on findings for publication in close collaboration with the Lab Director; assist in manuscript preparation for projects on which s/he was a collaborator; prepare and deliver presentations on his/her work at outside conferences; write fellowship proposals to bring funding to the lab; assist the Lab Director in writing larger grant proposals when requested. Maintain a high level of current knowledge in his/her discipline and in his/her specific field; maintain a high level of laboratory skills and continue to add new skills to his/her repertoire.

The Associate Scientist must also maintain university certifications for all necessary activities including human subjects, animal care and use, recombinant DNA, environmental and radiation safety, etc.

Minimum qualifications include a Ph.D. and/or M.D. with at least 5 years of work related experience. Experience preferred in ocular or CNS histology and transmission electron microscopy including specimen preparation, embedding, sectioning and immunogold labeling. Must have excellent English communication skills, both verbal and written. Any appropriate combination of relevant education, experience, and/or certifications will be considered.

[www.careers.med.miami.edu](http://www.careers.med.miami.edu)

EOE

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**Applications with clear reference to the position should  
be sent in printed form until February, 15<sup>th</sup>, 2010:**

University of Luxembourg • Professeur Paul Heuschling  
Dean of the Faculty of Science, Technology and Communication  
6, rue Coudenhove Kalergi • L-1359 Luxembourg

Please include all attachments as pdf-documents on a CD-ROM as well.

All applications will be handled in strictest confidence.

### Faculty Position in Marine Zooplankton Ecology Skidaway Institute of Oceanography

The Skidaway Institute of Oceanography (SkIO) invites applications for a faculty position in marine zooplankton ecology available 1 July 2010. This scientist will complement SkIO's existing programs in biological oceanography, microbial ecology, sediment and stable isotope geochemistry, coastal physics and sedimentary geology. We are particularly interested in applicants who study the role of macrozooplankton in trophic transfer, biogeochemical cycling, and ecosystem response to climate change. Applicants with proven interdisciplinary skills, oceanographic and experimental experience, and who employ novel approaches to these studies are especially encouraged. Applicants are encouraged to learn more about the Institute and its programs at [www.skio.usg.edu](http://www.skio.usg.edu).

The appointment will be made at either the Assistant or Associate Professor level, depending upon qualifications. Candidates should demonstrate strong evidence of (or potential for) the ability to conduct an active, extramurally funded research program. While SkIO is primarily a research institution, there are opportunities for interactions with local and regional academic institutions. Some level of participation in joint educational programs is expected.

Send CV, statement of research interests and teaching experience and contact information for at least three references to [resumes@skio.usg.edu](mailto:resumes@skio.usg.edu). Please attach required documents in PDF format. Electronic applications are strongly encouraged, but paper applications may be sent to **Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA, 31411**. Review of applications will begin on **March 1, 2010** and will continue until the position is filled.

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For further information please contact: [katerina@bodossaki.gr](mailto:katerina@bodossaki.gr)



### Assistant Commercial Editor Office of Publishing and Member Services

Publishing and Member Services is seeking a talented Assistant Commercial Editor to assist the Commercial Editor with the planning and execution of the feature articles on science technologies and careers as well as with other editorial tasks related to custom publications and webinars.

The position includes, but is not limited to, the following duties:

- Research and determine feature article topics; • Substantive editing of published material for content and style; • Assist the Commercial Editor in planning, soliciting, coordinating, and editing technology and career features (subjects are of a highly technical and specialized nature and require review by editorial staff); • Decide the placement and layout of published materials; • Plan and coordinate the Editorial calendar and production deadlines and track deadlines for feature articles; • Source suitable writers for feature articles and custom publications; • Handle all new products press releases, including choosing, researching, and editing/rewriting of releases; • Assist the Commercial Editor with planning and execution of custom publication projects, involving editing, writing, and research; • Stay abreast of new developments in the life science technology and science careers arenas, including visiting biotechnology suppliers onsite to learn about new technologies; • Attend domestic conferences as required; • Serve as the internal liaison with AAAS staff; • Track statistics and generate reports related to custom projects; and • Aid in the execution, tracking, and analysis of reader/user surveys.

The minimum qualifications to be competitive and considered for the position are:

- Extensive university or college-level training leading to a Master's degree or a Ph.D. (preferred) in the biological sciences; • Two to three years of related work experience with a Master's or one to two years of related work experience with a Ph.D.; • Strong proofreading and editing skills; • Excellent written and oral communication skills required in order to convey complex material and concepts; • Advanced computer skills; and • Experience in editing and in a publications setting preferred.

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# POSITIONS OPEN

## ASSISTANT/ASSOCIATE PROFESSOR/PROFESSOR Anatomical Sciences Medical College of Georgia

Department of Cellular Biology and Anatomy at the Medical College of Georgia, Augusta, invites applications for a full-time faculty position at the rank of Assistant/Associate, or full Professor. The successful candidate will take part in teaching gross anatomy and neuroanatomy. Candidates must have a Ph.D. and/or M.D. and have a minimum of two years of teaching experience in the anatomical sciences. Salary is dependent on qualifications and experience. The Medical College of Georgia is a state supported, comprehensive medical school whose mission is to train physicians and other health professionals to meet the health care needs of the state. The Department of Cellular Biology and Anatomy prides itself in state-of-the-art interdisciplinary research and excellence in education. Applicants should submit a letter with description of teaching interests and experience, curriculum vitae, and names of three references to [website: http://www.mcgc.edu/facultyjobs/](http://www.mcgc.edu/facultyjobs/). Review of applications will begin immediately and continue until the position is filled. **Requisition #4334.**

*The Medical College of Georgia is an Equal Employment Opportunity and Equal Access Institution.*

## UF UNIVERSITY of FLORIDA IFAS

### ASSISTANT PROFESSOR, Translational Genomics ASSISTANT/ASSOCIATE PROFESSOR, Plant Physiology

Two faculty positions (70 percent research, 30 percent teaching) are available in the Agronomy Department, Institute of Food and Agricultural Sciences, starting 1 July 2010. To view the complete position descriptions, qualifications, and application instructions, please visit [website: https://jobs.ufl.edu](https://jobs.ufl.edu) and search for **requisition #0803521 and #0803657**. Review of application materials will begin on or before 26 February 2010 and will continue until suitable applicants are identified.

*The University of Florida is an Equal Employment Opportunity Employer. Women and minorities are encouraged to apply.*

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# POSITIONS OPEN

## The University of Georgia

### DEPARTMENT HEAD Department of Genetics Franklin College of Arts and Sciences University of Georgia

The University of Georgia seeks an established scientist with creative vision to lead the Department of Genetics, [website: http://www.genetics.uga.edu](http://www.genetics.uga.edu). This search is coincident with a dramatic expansion campuswide of interdisciplinary research programs in the life sciences. With 25 faculty members, the Department has internationally recognized strengths in several areas of research, including molecular and developmental genetics; population and ecological genetics; and genome evolution and structure.

Candidates should have an outstanding record of academic accomplishments and funding, proven leadership and administrative skills, and a vision for future investment in the Department. Send applications or nominations electronically to **Dr. Stephen L. Hajduk, Chair of the Search Committee, e-mail: shajduk@bmb.uga.edu**. Applicants should submit a cover letter summarizing their qualifications and vision, together with a complete curriculum vitae. For full consideration, applications must be received by March 15, 2010, but the search will remain open until the position is filled.

*The Franklin College of Arts and Sciences, its many units, and the University of Georgia are committed to increasing the diversity of its faculty and students, and sustaining a work and learning environment that is inclusive. Women, minorities, and people with disabilities are encouraged to apply. The University is an Equal Employment Opportunity/Affirmative Action Institution.*

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